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ON THE RELATION OF BLOOD-VOLUME TO TISSUE-NUTRITION

IV. The Effects of Hemorrhage and Subsequent Intravenous Injection of Gum-Saline Solution on the Response of the Anesthetized Dog to the Alternate Administration of Room Air and of a Mixture of Carbon Dioxide in Room Air

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In paper I (1) of these studies on the relation of blood-volume to tissue-nutrition our methods failed to show a marked difference in the response of an unanesthetized animal to a progressive reduction in the percentage of oxygen in the respired air as a result of hemorrhage, even though hemorrhage elicited a definite reduction in the amount of oxygen consumed. Such results seemed to call for further experimentation along other lines. In these further experiments anesthesia was administered.

In the second study (2), employing striated muscle as a representative tissue, we found that hemorrhage and the injection of gum-saline solution respectively reduced and increased the volume-flow of blood out of proportion to the corresponding changes in blood-volume.

In the third series of experiments (3) in which we studied the effects of changes in blood-volume on the ability of striated muscle to respond to rapid electrical stimulation, we found that hemorrhage had detrimental effects. These effects varied with the degree of hemorrhage and also to a considerable extent with the particular animal employed.

[1] Preliminary report of this work will be found in the Proceedings of the Society for Experimental Biology and Medicine, xix, no. 1775.

Subsequent injection of a non-nutrient solution (gum-saline solution) invariably improved the response of the muscle.

The marked reduction in the total amount of oxygen consumed as a result of hemorrhage described in the first set of experiments (1) could not be definitely attributed to a nutritional disturbance resulting from a decrease in the volume-flow of blood for the reason already referred to in the original paper—that hemorrhage invariably produced a palpable softening of the muscles in the unanesthetized dog. A change in muscle tonus, other things remaining constant, might in itself affect the rate of oxygen consumption. As we felt that the loss or absence of muscle tonus resulting from anesthesia would prevent a further loss of tonus resulting from hemorrhage we performed additional and somewhat similar experiments on the anesthetized animal.

Two series of experiments were planned. In one series we attempted to demonstrate whether or not changes in blood-volume produced by hemorrhage and the injection of gum-saline solution, as employed in preceding experiments, affect the transport of gases such as carbon dioxide and oxygen. In the other series we attempted to determine whether or not similar hemorrhages and injections influence the gaseous metabolism-oxygen consumption. The results which we obtained on the relation of blood-volume to the transport of gases are described in this paper. The results on the relation of blood-volume to oxygen-consumption are described in the following paper of this series.

METHOD. The relation of blood-volume to the transport of gases was studied by observing the response of the anesthetized dog to the alternate administration of room air and of a 4 to 5 per cent mixture of carbon dioxide in room air. The method is comparable to that originally used by Loewy (4) to determine the effects of drugs upon the respiratory center. But in the present experiments carbon dioxide was administered not to study changes in the condition of the respiratory center, but rather, changes in the volume-flow of blood resulting from hemorrhages and the intravenous injection of gum-saline solution (6 per cent gum-acacia suspension in 0.9 per cent sodium chloride solution). We have worked on the assumption, how correctly we are not prepared to say as yet, that hemorrhage and the injection of gum-saline solution, barring the indirect effects of changes in the volume-flow of blood, exert no other marked effects upon the respiratory center. If that is true, we are justified in using the respiratory center as an indicator of changes in the carbon dioxide content of the blood associated with these changes in blood-volume.

Assuming that the formation of carbon dioxide in the tissues progresses at a constant rate and that ample time exists for the unloading of the major part of this carbon dioxide as the blood passes through the lungs, the more frequent the passage of blood from the point of loading to the point of unloading, the lighter the load of carbon dioxide as the blood leaves the tissues, and presumably the less is the respiratory center stimulated. Likewise, the lighter the load of carbon dioxide of the blood as it passes through the respiratory center, the less will be the total respiratory stimulus when the elimination of carbon dioxide is hampered by the administration of carbon dioxide. It would seem to follow that if the respiratory center serves as a reliable indicator of the "carbon dioxide ratio" of the blood, and if our assumption regarding the relation between the volume-flow and the carbon dioxide ratio of the blood is correct, we have a means of testing the effects of hemorrhage and of infusion of gum-saline solution upon the volume and nutrient-flow of blood.

The injection of gum-saline has two effects. It increases the volume-flow of blood and it dilutes the blood. This dilution of the blood necessarily entails a dilution of the carbon dioxide carriers. We, therefore, felt that if the injection of gum-saline solution which decreases the buffer properties of the blood still decreased the respiratory response to the administration of carbon dioxide, it improved the transport of carbon dioxide and lowered the carbon dioxide tension in the tissues through the improvement of the nutrient-flow. As will be discussed later, such a decrease in the respiratory response need not be due to a decrease in the flow of carbon dioxide carriers alone, but to the increased flow of oxygen as well. This point will receive further consideration in relation to the nature of the respiratory stimulus in a separate paper.

To administer room air and the carbon dioxide mixture the animal was connected by means of a tracheal cannula, an inspiratory and expiratory valve and a special arrangement of tubes with two Hutchinson spirometers of 50 liters capacity each. One spirometer was regularly filled with room air and the other with the carbon dioxide mixture. (A sufficient amount of this mixture was prepared in a large pressure tank fitted with a pressure gauge by running proper amounts of compressed air and carbon dioxide into the tank.) Manipulation of clamps on the rubber tubing connections permitted the alternate administration of the gases. The volume of gas respired was recorded by means of vertical writing points connected through a system of reducing pulleys with the top of the spirometers. Inspiration was

recorded by an upward stroke and expiration by a horizontal line. Respiration of constant ventilation was, therefore, represented by a line of steps of a constant gradient. (See fig. 1.)

Mean blood pressure and pulse rate were recorded with the mercury manometer. Time was recorded in seconds.

It was realized that anesthesia is a very important factor in the study of the respiratory function, and for that reason we considered employing decerebrate cats, but since the bulk of our work, serving as a basis for the present experiments, had already been done on the dog, we were led to continue our experiments on the same animal.

We soon found that the production of satisfactory anesthesia proved to be our greatest difficulty and undoubtedly accounted for some of the variability of our results. Experience led us to avoid the use of volatile anesthetics, such as ether, on account of the lack of control of anesthesia resulting from varying ventilation accompanying the alternate administration of room air and of the carbon dioxide mixture in room air. Morphine proved to be a valuable anesthetic due to the even anesthesia which it produced, but its well-known depressant action on the respiratory center limited its administration to relatively small amounts. The requisite anesthesia was obtained by the additional administration of urethane which was administered per rectum. As a rule approximately 0.15 cc. of a 2 per cent solution of morphine sulphate per kilogram of body weight was injected hypodermically about three-quarters of an hour before the experiment. This injection was followed in 15 to 20 minutes by the injection of 0.8 gram of urethane per kilogram of body weight. In some animals these injections produced an even anesthesia. In others further administration of either or both drugs was necessary.

In each experiment observations were begun only after allowing the animal to repeatedly empty the spirometer containing room air. This was done to determine whether or not the condition of the animal was constant. If each emptying of the spirometer produced straight gradients of similar pitch we began the alternate administration of room air and the carbon dioxide mixture. If they were not even, we frequently injected more anesthetic or allowed the anesthetics already injected a longer time to gain their effect.

Room air and the carbon dioxide mixture were administered at regular intervals. The duration of the administration of the carbon dioxide mixture varied in different experiments from 1 to $1\frac{1}{2}$ minutes. The administration of room air varied from $2\frac{1}{2}$ to 5 minutes. We felt that

this period permitted the elimination of most of the carbon dioxide accumulated during the preceding periods. After obtaining a series of two or three pairs of observations with the blood-volume normal, we repeated the observations after hemorrhage and again after the intravenous injection of gum-saline solution. After injection the animal was bled once more, and further observations with varying blood-volumes were continued as long as the condition of the animal warranted.

Results. We performed thirteen experiments, four of which were unsuccessful. The first experiment of the series was unsuccessful due to defective apparatus; the remaining three due to unsatisfactory anesthesia. In two of the nine remaining experiments the injection of gum-acacia proved toxic. The remaining seven show the effects of both hemorrhage and injection.

The nine experiments which yielded data are described in the order in which they were performed. The data of each experiment are tabulated. The tables show the number of the observation, the gas respired, ventilation in cubic centimeters per kilogram of body weight per minute, the difference in ventilation in cubic centimeters per kilogram of body weight per minute between the ventilations during the administration of the carbon dioxide mixture and of room air, the percentile difference of the same, the pulse rate per minute, the respiratory rate per minute, the mean blood pressure and the solar time. Hemorrhages and injections are indicated in per cent of body weight. Where several pairs of observations were made with each change in blood-volume, the average figures are given as well.

Figure 1 shows the procedure of experimentation and the type of record obtained. The lower tracing is the record of time in seconds. The second record shows the respiratory response to three separate administrations of carbon dioxide. The third record represents mean blood pressure. The upper record represents the respiratory response to the administration of room air. This figure was obtained in experiment 2 before hemorrhage. The difference in the gradients of the curves described by the two spirometers is discernible. The accurate determination of the ventilation is made as shown in the graph; the difference in length of the vertical lines subtending the respiratory records indicates the difference in ventilation. We call attention at this point to the progressive changes in the gradients at the beginning of the administration of either gas to a point where the gradient becomes practically constant.

Experiment 1. (*See table 1.*) Two pairs of observations (2 and 3, and 4 and 5) were obtained before hemorrhage. When the response

Fig. 1

to carbon dioxide in this experiment is compared with the response of the decerebrate cat (5) to a mixture of carbon dioxide of equal strength

it would appear that the anesthetics markedly lowered the irritability of the respiratory center. But as will be seen in later experiments, the depressant action of anesthetics was frequently much less marked. Following observations 2 to 5 the animal was bled to the extent of 1.09 per cent of the body weight and two more pairs of observations

TABLE 1

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
2	CO_2	398	126		172	26	110	11.54
3	air	275	123	46	232	22	108	
4	CO_2	385			242	26	106	
5	air	284	101	35	246	26	106	
av.	air	279						
av.	CO_2	391	112	40				
	Hemorrhage—95 cc.—1.09 per cent of body weight							
6	air	293			270	24	48	12.04
7	CO_2	471	178	60	292	28	48	
8	air	281			266	32	35	
9	CO_2	528	247	88	256	26	34	
av.	air	287						
av.	CO_2	499	212	74				
	Injection—250 cc.—2.89 per cent of body weight							
10	air	412			233	34	48	12.14
11	CO_2	553	141	34	270	42	53	
12	air	412			250	38	52	
13	CO_2	544	132	32	254	44	52	
av.	air	412						
av.	CO_2	548	136	33				

made (6 and 7, and 8 and 9). Inspection of column 3 of table 1 shows that hemorrhage exerted hardly any effect on the pulmonary ventilation during the administration of room air. The ventilation during the administration of carbon dioxide, however, was definitely increased, as shown in columns 3, 4 and 5. The hemorrhage eliciting these changes produced a large fall in blood pressure. We therefore injected gum-

TABLE 2

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	air	274			52	16	128	1.30
2	CO_2	471	197	72	52	18	134	
3	air	269			52	16	133	
4	CO_2	460	191	71	52	17	130	
5	air	269			52	16	132	
6	CO_2	481	212	79	54	18	134	
7	air	285			53	16	132	
av.	air	274						
av.	CO_2	471	197	72				
Hemorrhage—272 cc.—2.72 per cent of body weight								
8	air	274			152	16	109	1.52
9	CO_2	591	317	115	171	19	111	
10	air	285			142	16	99	
11	CO_2	596	311	109	168	20	103	
12	air	290			154	17	107	
13	CO_2	633	343	118	216	22	109	
av.	air	291						
av.	CO_2	606	315	108				
Injection 250 cc.—2.50 per cent body weight								
15	air	310			60	20	120	2.18
16	CO_2	575	265	85	50	20	122	
17	air	279			60	18	115	
18	CO_2	512	233	83	60	20	117	
19	air	290			62	18	116	
av.	air	293						
av.	CO_2	543	250	85				
Hemorrhage—225 cc.—2.25 per cent body weight 275 cc. were bled and 50 cc. of gum-saline solution promptly injected								
20	air	518			226	26	51	2.44
21	CO_2	1088	570	108	196	36	51	
22	air	455			208	24	55	
23	air	481			212	24	57	
24	CO_2	908	427	89	200	34	62	
25	air	497			216	28	55	
av.	air	488						
av.	CO_2	998	510	105				

TABLE 2—*Concluded*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Injection 250 cc.—2.50 per cent body weight								
26	air	372			204	24	94	3.11
27	CO_2	501	129	35	186	32	91	
28	air	378			202	24	86	
Hemorrhage—150 cc. (followed immediately by injection of 70 cc.). 0.75 per cent of body weight								
29	air	569			184	30	48	3.25
30	CO_2	1345	776	137	182	42	50	
Injection—200 cc.—2 per cent body weight								
31	air	388			190	28	78	3.36
32	CO_2	858	470	121	184	34	73	

saline solution rather promptly, but even though a large amount was administered (2.89 per cent of the body weight) the blood pressure failed to mount. Whether this was due to a toxic action of the gum-saline solution or to the fact that the animal was in the initial stages of shock as indicated by the serious effects of the previous hemorrhage, we cannot say. We give the data of two more pairs of observations after the injection. The increased ventilation of room air and of the carbon dioxide mixture resulting from injection differed from the usual response after the injection of gum-saline solution when the circulation appears to be improved.

Experiment 2. (*See table 2.*) Three pairs of observations (1 to 6) were obtained before hemorrhage. Attention has already been called to figure 1 which shows the smoked record of these observations. In this experiment, too, the extra ventilation elicited by the administration of the carbon dioxide mixture was not very large, yet the regularity of the response of this animal makes this experiment a very valuable one. If anesthesia prevents the full response of the animals to the administration of the carbon dioxide mixture under normal conditions, it may also prevent a maximum response after hemorrhage. The increased

response to carbon dioxide after hemorrhage may, therefore, be only a partial indication of the disturbance in the circulation produced by the decrease in blood-volume.

After observation 7, blood amounting to 2.72 per cent of the body weight was drawn. Only a relatively small fall in mean blood pressure occurred. Here, as in the preceding experiment, hemorrhage had little effect on the ventilation of room air,—291 cc. as compared with 274 cc. per kilogram of body weight per minute before hemorrhage (see column 3, table 2). The response to carbon dioxide, however, was decidedly increased from 471 cc. to 606 cc. per kilogram of body weight per minute. Observations 15 to 19 show that the injection of gum-saline solution had practically no effect upon room air ventilation (293 cc. as compared with 291 cc. per kilogram of body weight per minute after hemorrhage), but reduced the ventilation during the administration of carbon dioxide. The next hemorrhage of 2.25 per cent of the body weight, following observation 19, not only increased the response to carbon dioxide over that occurring after the first hemorrhage, but markedly increased the ventilation of room air. Compare the average figures of ventilation in column 3. Perhaps the large fall in blood pressure accounts for the increase in ventilation during the administration of room air, for mean blood pressure presumably is an important factor in the circulation of the brain. Observations 20, 21 and 22 after the second hemorrhage are shown in the right half of figure 2. At the left of the figure the effect of hemorrhage on the ventilation of room air is shown, in which a hemorrhage of 275 cc. produced a decided fall in the mean blood pressure and a marked dyspnea which showed no tendency to abate until 50 cc. of the lost blood-volume were replaced by the intravenous injection of 50 cc. of gum-saline solution. The effects of further injection of gum-acacia amounting to 2.5 per cent of the body weight on the response to room air and the carbon dioxide mixture are shown in observations 26 and 27. The hemorrhage following observation 28 produced almost identical results as those shown in figure 2. The same marked reduction in ventilation also accompanied the small injection of 70 cc. following immediately on the hemorrhage. Observations 29, 30, 31 and 32 show the effects on the response to alternate administration of room air and carbon dioxide as changed by the injection of gum-saline solution.

Experiment 3. (*See table 3.*) Three pairs of observations were obtained before hemorrhage. The average ventilations of room air and of the carbon dioxide mixture were 319 cc. and 504 cc. per kilogram of

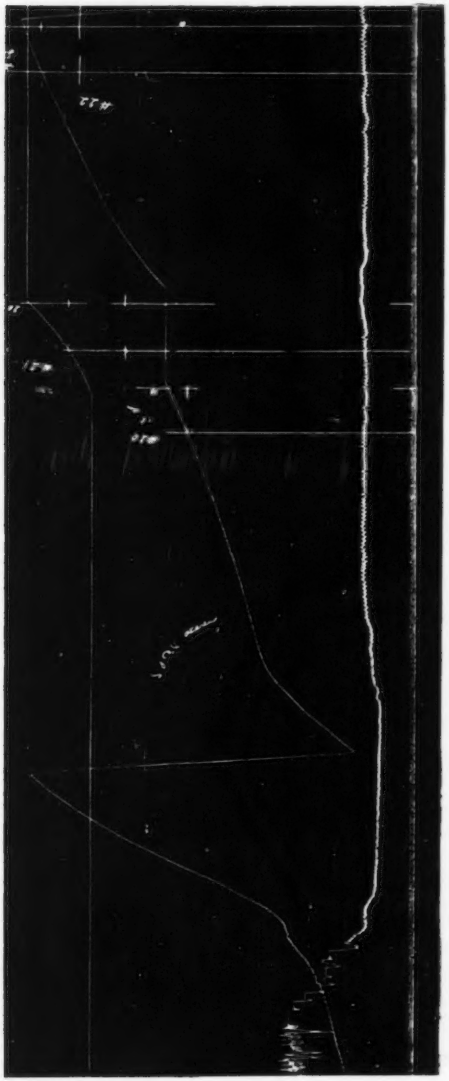

Fig. 2

body weight per minute respectively. A hemorrhage of 1.84 per cent of the body weight which lowered the blood pressure appreciably,

TABLE 3

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	air	325			130	30	100	10.55
2	CO_2	459	134	41	128	26	100	
3	air	306			132	26	100	
4	CO_2	545	239	78	130	26	98	
5	air	325			138	28	95	
6	CO_2	508	183	56	146	29	98	
7	air	319			146	30	96	
av.	air	319						
av.	CO_2	504	185	58				
	Hemorrhage—155 cc.—1.84 per cent body weight							
8	air	362			242	30	70	11.25
9	CO_2	620	258	71	226	48	72	
10	air	411			232	30	62	
11	CO_2	551	140	34	222	34	64	
12	air	441			242	32	65	
13	CO_2	601	160	36	228	40	68	
14	air	472			206	35	66	
av.	air	421						
av.	CO_2	591	170	40				
	Injection—170 cc.—2.01 per cent body weight							
15	air	454			162	50	76	11.44
16	air	424			192	42	77	
17	CO_2	620	191	44	194	46	81	
18	air	308			202	38	80	
19	CO_2	570	262	85	194	42	78	
av.	air	397						
av.	CO_2	595	198	50				

increased the ventilation of both gas mixtures. Inspection of the second last column shows a tendency toward a progressive fall in mean blood pressure, and we note that the injection of gum-saline solution after

observation 14 raised the pressure very little. Perhaps this accounts for the small changes in the pulmonary ventilation resulting from the increased blood-volume.

Experiment 4. (*See table 4.*) Inspection of column 3 of table 4 shows that the first hemorrhage amounting to 1.57 per cent of the body weight elicited an average reduction in the ventilation of room air and of the carbon dioxide mixtures. This is the only instance of its kind occurring at the beginning of an experiment, and we are unable to offer an explanation, but merely point out that the gradual decrease in pulse rate and respiratory rate during observations 1 to 10 indicates some progressive change in the animal up to the first hemorrhage. The second hemorrhage (observations 23 to 32) increased the ventilation of room air from 191 cc. to 218 cc. per kilogram of body weight per minute, and the ventilation of the carbon dioxide mixture from 237 cc. to 256 cc. per kilogram of body weight per minute. Injection of gum-saline solution to the extent of 1.49 per cent of the body weight (observations 33 to 41) decreased the ventilation of room air from 218 cc. to 196 cc. and of the carbon dioxide mixture from 256 cc. to 230 cc. per kilogram of body weight per minute. A second injection to the extent of 0.74 per cent of the body weight lowered the ventilation still more—of room air from 196 cc. to 176 cc. and of the carbon dioxide mixture from 230 cc. to 214 cc. per kilogram of body weight. The two following hemorrhages (observations 50 to 58 and 59 to 67) and the subsequent injection produced respectively an increase and a decrease in the ventilation of both room air and of the carbon dioxide mixture. The last hemorrhage (observations 77 to 83) produced a decrease in the ventilation of room air and of the carbon dioxide mixture. The ventilation of room air was reduced from 223 cc. to 197 cc. and of the carbon dioxide mixture from 253 cc. to 222 cc. per kilogram of body weight per minute. Subsequent injection of gum-saline solution (observations 84 to 92) slightly increased the ventilation of both gas mixtures. The reduction in ventilation associated with the last hemorrhage, we believe, is due to the rapid deterioration of the animal resulting from the critical disturbance in the circulation indicated by the low mean blood pressure. The increase in ventilation associated with the injection of gum-saline solution may be explained by the improvement of the circulation through the respiratory center which permitted a slightly greater response. Our interpretation is borne out by the progressive decrease in heart rate following the last hemorrhage and the progressive increase in heart rate following the last injection.

TABLE 4

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	air	189			136	40	116	11.00
2	CO_2	269	80	42	126	56	116	
3	air	259			122	54	112	
4	CO_2	324	65	25	114	54	108	
5	air	205			110	32	110	
6	CO_2	343	138	67	126	54	110	
7	air	239			110	42	111	
8	CO_2	335	96	40	110	50	112	
9	air	220			126	22	114	
10	CO_2	327	107	49	90	30	113	
11	air	232			106	34	116	12.19
av.	air	224						
av.	CO_2	319	95	42				
Hemorrhage—2.10 cc.—1.57 per cent body weight								
12	air	224			102	22	94	
13	CO_2	265	41	18	102	32	86	
14	air	193			118	24	78	
15	CO_2	242	49	25	126	25	76	12.35
16	air	178			126	20	76	
17	CO_2	217	39	22	134	22	78	
18	air	185			144	20	79	
19	CO_2	217	32	17	140	20	80	
20	air	181			146	20	82	
21	CO_2	242	61	34	140	22	80	
22	air	185				22	82	12.52
av.	air	191						
av.	CO_2	237	46	24				
Hemorrhage—87 cc.—0.65 per cent body weight								
23	air	170			152	20	79	1.05
24	air	208			202	26	66	
25	CO_2	234	26	12	182	24	64	
26	air	200			210	24	60	
27	CO_2	261	61	30	192	26	62	
28	air	228			196	30	60	
29	CO_2	265	37	16	194	28	60	

TABLE 4—*Continued*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Hemorrhage—87 cc.—0.65 per cent body weight—Continued								
30	air	243			222	32	60	
31	CO_2	265	22	9	212	32	59	
32	air	263			206	34	56	1.24
av.	air	218						
av.	CO_2	256	38	17				
Injection—200cc.—1.49 per cent body weight								
33	air	197			134	27	79	
34	CO_2	277	80	40	130	26	78	
35	air	205			140	25	76	
36	CO_2	230	25	12	136	24	78	
37	air	193			136	21	80	
38	CO_2	203	10	5	132	20	81	
39	air	197			132	21	83	
40	CO_2	211	14	7	144	22	84	
41	air	189			150	20	87	1.58
av.	air	196						
av.	CO_2	230	34	17				
Injection—100 cc.—0.74 per cent body weight								
42	air	170			138	18	96	
43	CO_2	207	37	22	140	20	97	
44	air	185			150	22	96	
45	CO_2	222	37	20	140	22	98	
46	air	185			144	21	100	
47	CO_2	232	47	25	140	22	99	
48	air	166			144	20	99	
49	CO_2	194	28	17	140	20	101	2.17
av.	air	176						
av.	CO_2	214	38	22				
Hemorrhage—200 cc.—1.49 per cent body weight								
50	air	200			232	24	77	2.25
51	CO_2	214	14	7	236	22	83	
52	air	193			216	22	80	

TABLE 4—*Continued*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Hemorrhage—200 cc.—1.49 per cent body weight—Continued								
53	CO_2	226	33	17	222	24	80	
54	air	193			220	23	76	
55	CO_2	254	61	32	212	24	76	
56	air	193			210	22	78	
57	CO_2	222	29	15	214	22	80	
58	air	200			252	24	77	2.50
av.	air	196						
av.	CO_2	226	30	15				
Hemorrhage—125 cc.—0.93 per cent body weight								
59	air	224				28	44	
60	CO_2	250	26	12		28	52	
61	air	239				28	54	
62	CO_2	269	30	13		27	58	
63	air	232				28	56	
64	CO_2	261	29	13		28	62	
65	air	239			240	29	58	
66	CO_2	269	30	13	218	28	61	
67	air	251			212	30	50	3.15
av.	air	237						
av.	CO_2	262	25	11				
Injection—300 cc.—2.24 per cent body weight								
68	CO_2	257			210	28	87	
69	air	228	29	11	206	28	90	
70	CO_2	250			210	26	90	
71	air	224	26	10	192	26	91	
72	CO_2	250			196	27	93	
73	air	216	34	14	210	26	94	
74	CO_2	257			212	26	95	
75	air	224	33	13		28	94	
76	air	224			222	25	91	3.41
av.	air	223						
av.	CO_2	253	30	13				

TABLE 4—*Concluded*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	CHE
Hemorrhage—300 cc.—2.24 per cent body weight								
77	air	170			246	30	31	
78	CO_2	194	24	14	196	34	36	
79	air	212			206	34	37	
80	CO_2	234	22	10	208	34	37	
81	air	209			196	37	36	
82	CO_2	237	28	13	194	36	36	
83	air	179			182	30	32	4.10
av.	air	197						
av.	CO_2	222	25	13				
Injection—315 cc.—2.35 per cent body weight								
84	air	212			156	17	54	
85	CO_2	237	25	12	178	16	56	
86	air	204			192	18	62	
87	CO_2	211	7	3	196	18	66	
88	air	220			188	22	69	
89	CO_2	237	17	8	196	23	73	
90	air	247			196	26	70	
91	CO_2	218			196	24	73	
92	air	224			218	26	70	4.47
av.	air	221						
av.	CO_2	226	5	2				

Experiment 5. (*See table 5.*) The results of this experiment are very striking. The first hemorrhage amounting to only 1 per cent of the body weight and producing only a relatively small fall in the mean blood pressure elicited a marked response. Though the ventilation of room air was increased from 596 cc. to only 611 cc. per kilogram of body weight per minute, the ventilation of the carbon dioxide mixture was increased from 897 cc. to 2030 cc. per kilogram of body weight per minute. Replacement of the lost blood by gum-saline solution (observations 17–23) reduced the ventilation of room air to 589 cc. and of the carbon dioxide mixture to 1155 cc. per kilogram of body weight per minute. Observations 6 and 7 before hemorrhage, 13 and 14 after hemorrhage, and 17 and 18 after injection are shown in figure 3. Sub-

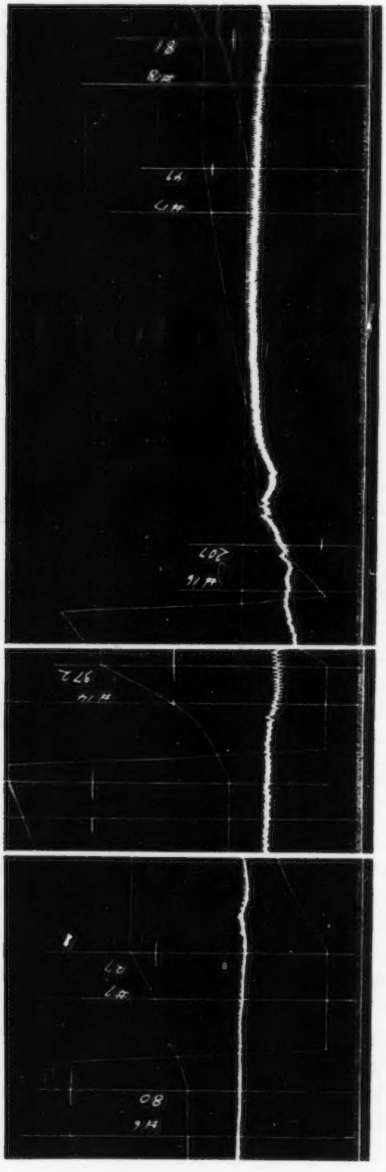

Fig. 3

sequent hemorrhage (observations 24 and 25) amounting to 0.97 per cent of the body weight raised the ventilation of room air considerably, and the administration of the carbon dioxide mixture produced such marked dyspnea that we were forced to shorten its administration, consequently the exact measurement of ventilation was unobtainable. Had we continued the administration of carbon dioxide the usual period of time, the increase in ventilation over that of room air undoubtedly would have been several hundred per cent. Injection again (observations 26 to 31) produced a striking reduction in the ventilation of room air and of the carbon dioxide mixture. The next hemorrhage (observations 32 to 36) produced the usual increase in ventilation, but the following hemorrhage (observations 37 to 42) reduced the respiratory

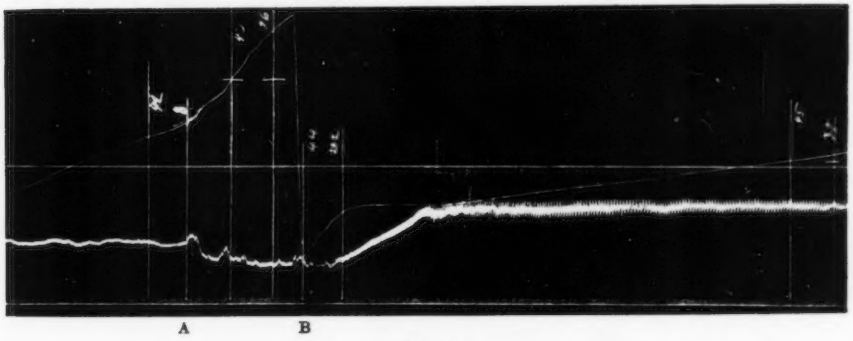

Fig. 4

response. Whether this slight reduction in ventilation can be attributed to a slight deterioration or to a reduced irritability of the respiratory center is not so certain as our conclusion regarding comparable results in the preceding experiment. The following injection (observations 45 to 52) and hemorrhage (observations 53 to 54) elicited again the decrease and increase in the respiratory response.

Figure 4 obtained from this experiment illustrates the marked effects which the rate of flow of blood exerts upon respiration. The record shows the ventilation of room air. At the left the blood-volume of the animal was below normal and associated with this condition there was a relatively rapid ventilation. At point *A* blood samples amounting to 12 cc. were taken. As a result of this small reduction in the blood-volume the ventilation was increased. At *B* the spirometer was refilled and very shortly after the blood-volume was quickly increased by the

TABLE 5

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	air	952			158	88	138	10.20
2	air	509			170	56	124	
3	CO_2	772	263	52	158	64	116	
4	air	615			162	58	119	
5	CO_2	982	367	60	158	74	119	
6	air	598			168	62	123	
7	CO_2	952	354	59	152	82	121	
8	air	637			170	62	122	
9	CO_2	884	257	40	158	72	124	
10	air	622			164	62	124	
av.	air	596						
av.	CO_2	897	301	51				
	Hemorrhage—70 cc.—1.00 per cent body weight							
11	air	607			138	60	92	11.04
12	CO_2	1274	667	110	168	78	104	
13	air	615			126	58	98	
14	CO_2	2787	2172	353	148	130	88	
av.	air	611						
av.	CO_2	2030	1419	232				
	Injection—70 cc.—1.00 per cent body weight							
17	air	352			150	32	116	
18	CO_2	607	255	72	142	37	111	
19	air	645			144	56	112	11.26
20	CO_2	1479	837	129	162	86	116	
21	air	622			164	54	120	
22	CO_2	1380	768	123	158	80	120	
23	air	712			166	66	110	
av.	air	589						
av.	CO_2	1155	571	98				
	Hemorrhage—68 cc.—0.97 per cent body weight							
24	air	1162			130	84	64	11.48
24a	CO_2	Marked dyspnea						
25	air	1334		Several hundred	150	104	71	

TABLE 5—*Continued*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Injection—100 cc.—1.43 per cent body weight								
26	air	352			116	34	106	
27	air	646			128	56	98	
28	CO_2	1166	520	80	150	76	98	
29	air	509			110	52	102	
30	CO_2	1559	1050	226	156	90	100	
31	air	499			110	54	100	12.23
av.	air	501						
av.	CO_2	1362	861	172				
Hemorrhage—70 cc.—1.00 per cent body weight								
32	air	502			170	64	72	
33	CO_2	1678	1176	234	164	78	84	
34	air	741			188	88	82	
35	CO_2	1569	910	123	170	70	104	
36	air	659			170	78	76	12.35
av.	air	634						
av.	CO_2	1623	989	156				
Hemorrhage—43 cc.—0.61 per cent body weight								
37	air	1638			172	96	44	
38	air	524			164	76	60	
39	CO_2	1424	900	172	154	70	76	
40	air	517			174	60	74	
41	CO_2	1568	1051	203	164	74	73	
42	air	615			168	66	62	
av.	air	552						
av.	CO_2	1496	944	180				
Injection—145 cc.—2.07 per cent body weight								
45	air	277			136	30	102	
46	air	298			144	35	108	
47	CO_2	682	384	129	134	48	100	
48	air	322			182	34	105	
49	CO_2	495	173	54	136	36	105	
50	air	427			128	42	96	

TABLE 5—*Concluded*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Injection—145 cc.—2.07 per cent body weight—Concluded								
51	CO_2	1124	697	163	122	59	96	
52	air	555			154	52	84	1.20
av.	air	376						
av.	CO_2	767	391	104				
Hemorrhage—65 cc.—0.93 per cent body weight								
53	air	600			122	54	71	
54	CO_2	1296	696	116	118	60	90	

injection of gum-saline solution. This injection produced an apnea lasting about one minute which was followed by a period of decreased respiratory volume.

In this experiment the effect of administering gum-saline solution during the maximum respiratory response to the administration of the carbon dioxide mixture was tried. The injection markedly reduced the ventilation.

Experiment 6. (*See table 6.*) In this experiment hemorrhage (observations 13 to 21) increased the ventilation of room air and of the carbon dioxide mixture. Subsequent injection (observations 21 to 32) elicited a peculiar response in this instance in that it reduced the response to the administration of room air and increased the response to the carbon dioxide mixture.

Experiment 7. (*See table 7.*) The effects of two successive hemorrhages were observed. The first hemorrhage amounting to 1.41 per cent of the body weight elicited practically no change in the respiratory response. The second hemorrhage amounting to 0.74 per cent of the body weight increased the ventilation of room air and of carbon dioxide. No results on the effects of increasing the blood-volume were obtained. The injection of gum-saline solution proved toxic.

Experiment 8. (*See table 8.*) Up to observation 40 the results obtained were consistent with the usual response noted in the preceding experiments. A hemorrhage amounting to 1.02 per cent of the body

TABLE 6

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	air	286			208	22	100	2.40
2	CO_2	533	247	86	184	23	101	
3	air	286			206	18	101	
4	CO_2	533	247	86	182	22	104	
5	air	286			188	14	101	
6	CO_2	501	215	75	190	20	100	
7	air	254			204	18	100	
8	CO_2	541	287	113	184	20	100	
9	air	222			192	22	100	
10	CO_2	477	255	115	198	22	103	
11	air	366			194	26	101	
12	CO_2	556	190	52	204	20	103	3.21
av.	air	283						
av.	CO_2	525	242	85				
Hemorrhage—65 cc.—0.98 per cent body weight								
13	air	477			192	42	69	
14	CO_2	644	167	35	196	36	69	
15	air	485			188	40	54	
16	CO_2	636	151	31	182	34	66	
17	air	421			182	32	58	
18	CO_2	700	279	66	190	34	65	
19	air	485			180	38	56	
20	CO_2	587	102	21	182	30	64	
21	air	469			184	30	61	
av.	air	467						
av.	CO_2	642	175	37				
Injection—90 cc.—1.36 per cent body weight								
21a	air	334			150	23	97	
22	CO_2	620	286	86	128	20	101	3.45
23	air	302			162	23		
24	CO_2	787	485	161	138	22	106	
25	air	294			166	20	127	
26	CO_2	771	477	162	150	21	127	
27	air	334			178	18	127	
28	CO_2	912	578	173	142	22	132	

TABLE 6—*Concluded*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Injection—90 cc.—1.36 per cent body weight—Continued								
29	air	318			192	20	112	
30	CO_2	1583	1265	398	160	17	124	
31	air	423			182	26	116	
32	CO_2	859	436	103	138	20	116	
av.	air	334						
av.	CO_2	922	588	176				

TABLE 7

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
av.	air	265			152	29	121	10.15
av.	CO_2	342	77	29	148	31	124	
Hemorrhage—120 cc.—1.41 per cent body weight								
av.	air	276			146	31	96	
av.	CO_2	361	85	31	140	31	98	
Hemorrhage—63 cc.—0.74 per cent body weight								
av.	air	355			140	38	69	
av.	CO_2	410	55	15	146	38	73	10.47

weight increased the ventilation of room air from 333 cc. to 603 cc. and of the carbon dioxide mixture from 496 cc. to 1015 cc. per kilogram of body weight per minute. Injection reduced the ventilation of room air and the carbon dioxide mixture to 542 cc. and 751 cc. and hemorrhage increased these values again to 608 cc. and 908 cc. per kilogram of body weight per minute respectively. The next injection (observa-

TABLE 8

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE BODY	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	air	297			160	33	108	10.40
2	CO_2	408	111	37	140	31	108	
3	air	328			152	37	106	
4	CO_2	457	129	39	146	36	110	
5	air	359			202	46	96	
6	CO_2	538	179	50	148	44	102	
7	air	347			162	46	94	
8	CO_2	581	234	67	160	48	98	
av.	air	333						
av.	CO_2	496	163	49				
	Hemorrhage—87 cc.—1.02 per cent body weight							
9	air	421			184	40	76	11.23
10	CO_2	768	347	82	120	41	86	
11	air	508			150	44	83	
12	CO_2	970	462	91	108	54	92	
13	air	650			106	58	93	
14	CO_2	1115	465	72	90	72	98	
15	air	670			100	68	85	
16	CO_2	1115	445	68	106	74	92	
17	air	768			168	70	76	
18	CO_2	1158	390	51	128	80	92	11.50
av.	air	603						
av.	CO_2	1015	412	68				
	Injection—110 cc.—1.29 per cent body weight							
19	air	638			174	66	98	
20	CO_2	956	318	50	152	68	104	
21	air	601			202	58	102	
22	CO_2	867	266	44	128	60	108	
23	air	611			164	52	100	
24	CO_2	809	198	32	192	53	104	
25	air	550			158	48	98	
26	CO_2	722	172	31	160	48	100	
27	air	494			180	47	92	12.20
28	CO_2	735	241	49	158	44	96	

TABLE 8—*Continued*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Injection—110 cc.—1.29 per cent body weight—Continued								
29	air	512			194	42	84	
30	CO_2	741	229	45	150	46	90	
av.	air	542						
av.	CO_2	751	209	39				
Hemorrhage—86 cc.—1.01 per cent body weight								
31	air	581			170	48	40	
32	CO_2	896	315	54	150	50	44	
33	air	630			156	48	38	
34	CO_2	914	284	45	140	46	48	
35	air	593			142	40	42	
36	CO_2	926	333	56	148	44	52	
37	air	630			160	42	48	
38	CO_2	889	259	41	138	40	56	1.00
av.	air	608						
av.	CO_2	908	300	49				
Injection—110 cc.—1.29 per cent body weight								
40	air	550			176	41	94	
41	CO_2	908	358	65	194	40	98	
42	air	618			190	41	98	
43	CO_2	933	315	51	180	40	102	
44	air	618			172	40	96	
45	CO_2	908	290	47	148	44	104	
46	air	601			188	39	98	
47	CO_2	938	337	56	154	40	102	1.27
av.	air	668						
av.	CO_2	921	253	38				
Hemorrhage—43 cc.—0.51 per cent body weight								
48	air	557			164	43	76	
49	CO_2	1083	526	94	124	49	82	
50	air	575			144	42	82	
51	CO_2	982	407	71	138	44	86	

TABLE 8—*Concluded*

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Hemorrhage—43 cc.—0.51 per cent body weight—Continued								
58	air	601			150	43	88	
59	CO_2	935	334	56	146	44	92	
av.	air	578						
av.	CO_2	990	412	71				
Injection—63 cc.—0.74 per cent body weight								
60	air	508			188	42	99	1.55
61	CO_2	867	359	71	168	44	102	
62	air	532			190	39	102	
63	CO_2	855	323	61	140	42	108	
64	air	520			170	38	106	
65	CO_2	805	285	55	168	41	108	2.15
av.	air	520						
av.	CO_2	842	322	62				

tions 40 to 47) instead of decreasing the respiratory response, increased it. Aside from this response and the decrease in the ventilation of room air following the subsequent hemorrhage (observations 48 to 59), the remaining responses followed the rule.

Experiment 9. (*See table 9.*) The average figures for ventilation of room air and of the carbon dioxide mixtures show a consistent response of the animal to changes in blood-volume. A decrease in blood-volume increased the ventilation of room air, and of the carbon dioxide mixture, and an increase in blood-volume had the reverse effect. Note that this animal was exceedingly sensitive to changes in blood-volume. A hemorrhage of only 0.32 per cent of the body weight (observations 9 to 16) almost doubled the ventilation of both gases, though its effect, if any, upon the mean blood pressure was to slightly elevate it. Exact replacement of the lost blood with gum-saline solution (observations 17 to 22) produced equally striking effects. The response after injection returned practically to the initial values. A second but larger hemorrhage (observations 23 to 28) elicited greater

TABLE 9

OBSERVATION	RESPIRING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	air	461			210	20	106	10.17
2	CO_2	1109	648	140	196	24	110	
3	air	546			206	20	109	
4	CO_2	1041	495	91	186	24	110	
5	air	705			202	22	108	
6	CO_2	1310	605	86	182	26	116	
7	air	815			222	24	110	
8	CO_2	1461	646	79	160	29	117	
av.	air	632						
av.	CO_2	1230	598	95				
Hemorrhage—20 cc.—0.32 per cent body weight								
9	air	1092				104	106	10.42
10	CO_2	1787	595	55		66	114	
11	air	1050				74	108	
12	CO_2	1940	890	85		72	116	
13	air	697				31	112	
14	CO_2	1477	780	112		38	116	
15	air	1184				88	118	
16	CO_2	2150	966	82		70	122	
av.	air	1006						
av.	CO_2	1838	832	78				
Injection—20 cc.—0.32 per cent body weight								
17	air	571			206	31	116	11.03
18	CO_2	1000	429	75	192	33	116	
19	air	789			172	32	118	
20	CO_2	1225	436	55	162	35	118	
21	air	789			192	28	118	
22	CO_2	1485	696	88	156	37	122	
av.	air	716						
av.	CO_2	1236	520	73				
Hemorrhage—51 cc.—0.82 per cent body weight								
23	air	1108				284	113	11.24
24	CO_2	2024	916	82			120	

TABLE 9—*Concluded*

OBSERVATION	BREATHING AIR OR CO_2 MIXTURE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	DIFFERENCE IN VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PERCENTAL DIFFERENCE OF VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	PULSE RATE PER MINUTE	RESPIRATORY RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Hemorrhage—51 cc.—0.82 per cent body weight—Continued								
25	air	1477				288	112	
26	CO_2	2369	892	60			122	
27	air	1494				288	112	
28	CO_2	1948	454	30			122	
av.	air	1359						
av.	CO_2	2113	754	55				
Injection—93 cc.—1.49 per cent body weight								
29	air	663			168	20	126	11.41
30	CO_2	1100	437	66	172	29	123	
31	air	756			166	22	132	
32	CO_2	992	236	31	162	24	130	
33	air	840			190	27	130	
34	CO_2	1158	318	38	174	26	130	
av.	air	753						
av.	CO_2	1083	330	44				
Hemorrhage—54 cc.—0.86 per cent body weight								
35	air	1000			190	61	124	12.02
36	CO_2	2040	1040	104		34	128	
37	air	1452			160	100	120	
38	CO_2	1890	438	30		52	123	
39	air	1049			170	68	122	
av.	air	1166						
av.	CO^1	1965	799	68				
Injection—75 cc.—1.20 per cent body weight								
40	air	557			186	21	126	12.16
41	CO_2	945	388	70	176	25	123	
42	air	840			204	25	122	
43	CO_2	1412	572	68	198	32	126	
44	air	934			210	29	126	
45	CO_2	1712	778	83	176	34	128	
av.	air	790						
av.	CO_2	1437	799	101				

responses. The pulmonary ventilations after the first and second hemorrhage during the administration of room air were respectively 1006 cc. and 1359 cc. per kilogram of body weight per minute. The ventilations during the administration of the carbon dioxide mixture were 1838 cc. and 2113 cc. per kilogram of body weight per minute. When the blood-volume (observations 29 to 34) was increased above any preceding volume the response to the administration of the carbon dioxide mixture was less than any preceding response. The point to which we wish to call particular attention in this experiment is the fact that the hemorrhages were small, that they showed no tendency to produce a fall in blood pressure, and yet elicited a marked increase in the respiratory response which was removed by the replacement of the lost blood-volume with gum-saline solution.

SUMMARY AND CONCLUSIONS

The effects of hemorrhage and of the intravenous injection of gum-saline solution on the respiratory response of the anesthetized dog to the alternate administration of room air and of a mixture of carbon dioxide in room air were studied.

The object of the experiments was to determine the effects of hemorrhage and subsequent injection of a non-nutrient solution on the transport of blood gases.

The results of nine experiments are reported.

In these experiments we found that the pulmonary ventilation during the administration of carbon dioxide was greater after hemorrhage than during the administration of same mixture before hemorrhage.

The effects of hemorrhage on pulmonary ventilation during the administration of room air varied considerably. In some instances large hemorrhage had little or no effect on the pulmonary ventilation of room air and in other instances small hemorrhage markedly increased the ventilation.

Injection of gum-saline solution subsequent to hemorrhage decreased the respiratory response to the administration of the carbon dioxide mixture.

In those instances in which hemorrhage increased the pulmonary ventilation of room air the injection of gum-saline solution decreased this ventilation.

The changes in the respiratory response elicited by hemorrhage and injection varied with the extent of the changes in blood-volume.

Hemorrhage and injection which elicited the usual respiratory re-

sponses not infrequently were accompanied by no or small alterations in the mean blood pressure.

In one instance hemorrhage and subsequent injection each amounting to only 0.32 per cent of the body weight increased and decreased the respiratory response to the administration of carbon dioxide from 1230 cc. to 1836 cc. to 1236 cc. per kilogram of body weight per minute. These changes occurred in the absence of a respective decrease and increase in the mean blood pressure.

The results appear to indicate that hemorrhage elicited a disturbance in the normal transport of carbon dioxide and that the injection of gum-saline solution improved this transport.

The disturbance in the transport of carbon dioxide after hemorrhage is ascribed to a reduced volume-flow of blood; the improvement in transport following injection to an increased volume-flow of blood.

Since the intravenous injection of gum-saline solution dilutes the carbon dioxide carriers of the blood and therefore decreases the buffer value of the blood, and since injection leads to a decrease rather than an increase in the respiratory response, we conclude that the injection of this non-nutrient solution increased the nutrient-flow by increasing the volume-flow of blood out of proportion to the dilution of the blood entailed by the injection.

The changes in the respiratory response resulting from hemorrhage and injection may be attributable to changes in the transport of carbon dioxide alone, but since a reduction of oxyhemoglobin to reduced hemoglobin increases the buffer value of the blood, we are inclined to consider the significance of changes in the transport of oxygen as well.

Lack of oxygen may indirectly stimulate the respiratory center by limiting the extent of the reduction of oxyhemoglobin, thereby increasing the effectiveness of the acid metabolites added to the blood in stimulating the respiratory center. Consequently if the changes in the blood volume occurring in these experiments affect the transport of oxygen sufficiently to alter the rate of oxygen consumption, we suggest that the accompanying changes in the respiratory response are due to alterations in the transport of oxygen as well as to alterations in the transport of carbon dioxide.

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ON THE RELATION OF BLOOD-VOLUME TO TISSUE-NUTRITION

V. The Effects of Changes in Blood-Volume Elicited by Hemorrhage and the Intravenous Injection of Gum-Saline Solution on the Total Oxygen Consumption of the Anesthetized Dog

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In the preceding paper (1) on the effects of hemorrhage and of the intravenous injection of gum-saline solution on the response to the alternate administration of room air and of a mixture of carbon dioxide in room air, we have demonstrated that a relatively small hemorrhage may increase the pulmonary ventilation during the administration of a mixture of carbon dioxide in room air, and also, at times, of room air alone. The replacement of the lost blood by a non-nutrient solution such as a 6 per cent suspension of gum-arabic in a 0.9 per cent solution of sodium chloride reduced the response to the administration of both gases. We believe that the increased respiratory response associated with hemorrhage is a result of a disturbance in the normal transport of blood-gases entailed by a decrease in the volume-flow of blood. We also believe that the decreased respiratory response associated with intravenous injection of gum-saline solution is due to an improvement in the transport of gases resulting from the acceleration in the volume-flow of blood which is out of proportion to the dilution of the blood entailed by such injection.

Though the results which we obtained presumably might be explained by the alteration of the transport of carbon dioxide alone, yet the close dependence of both the loading of the blood with carbon dioxide and the hydrogen ion concentration of the blood on the extent of the synchronous unloading of oxygen, inclined us to attribute part of the increased respiratory response to a disturbance in the transport of oxygen as well.

[1] Preliminary report may be found in the Proceedings of the Society for Experimental Biology and Medicine, 1921–22, xix, no. 1776.

In the present series of experiments we attempted to determine whether or not the disturbance of the transport of oxygen entailed by hemorrhage reduced the total oxygen consumption and whether or not the replacement of the lost blood by a non-nutrient solution had any effect. The results, however, have another interest in relation to the mechanism of the stimulating effect of the lack of oxygen on the respiratory center.

METHOD. At this point we wish to call attention to data obtained in the first series (2) of these experiments on the unanesthetized dog, on the effects of hemorrhage on the respiratory and circulatory response to a progressive diminution in the amount of oxygen in the respired air. We found in these experiments that hemorrhage produced a marked reduction in the rate of oxygen consumption, due, however, to the fact that hemorrhage in the unanesthetized dog produced a general quieting effect and elicited a loss of muscle tonus as indicated by the general palpable softening of the muscles, we were at a loss to know how much, if any, the reduction in the oxygen consumption was due to a reduced flow of blood or whether it was caused by a reduction in the tonus of the striated muscle. For that reason, as was pointed out before, further experiments were performed on the dog under anesthesia in which changes in blood-volume presumably would elicit no changes in muscle tonus.

Anesthesia was produced as described in the preceding paper. Mean blood pressure and time were recorded as usual.

A modification of the direct method of determining the rate of oxygen consumption employed in the portable respiration apparatus described by Benedict (3) was used. The rate of consumption was graphically recorded by connecting the dog by means of a tracheal cannula with a Henderson rebreathing apparatus fitted with a cartridge of soda-lime for the absorption of the carbon dioxide of the expired air. The cartridge was of large bore and was fitted with coarse soda-lime (4 mesh) which offered very little resistance to expiration. The soda-lime was replenished at regular intervals to insure complete absorption of the carbon dioxide. The rebreathing apparatus was equipped with an accurately calibrated spirometer which served to record the rate and volume of respiration and the rate of oxygen consumption. Records similar to figure 1 were obtained. Inspiration is represented by a down-stroke and expiration by an up-stroke. As the oxygen is consumed the total volume of gas in the rebreathing apparatus diminishes and the general level of the respiratory record progressively falls with each respiration.

In many experiments the respiration occurred with perfect regularity. The upper and lower ends of a series of respirations were perfectly aligned by two parallel lines of equal gradients (see fig. 1A). In some experiments this did not hold. Irregularities occurred in either or both lines; but of the two lines which join the ends and beginnings of a series of respirations the first or the upper line was as a rule the more regular (see fig. 1B). This line, which we will call the line of expiratory rest, was used to determine the rate of oxygen consumption. The drop of the line from the horizontal in a period of 2 to 4 minutes served as our unit of measurement. All measurements were reduced to oxygen consumption in cubic centimeter per kilogram of body weight per minute. Figure 2 is a sample record showing the effect of injection on the rate of oxygen consumption. Note the change in the gradient of the line of expiratory rest.

The procedure in all experiments was to allow 3 liters of room air per kilogram of body weight in the rebreathing apparatus. The apparatus was adjusted so that the spirometer was at its highest position at the beginning of the experiment. The dog was allowed to rebreathe the air until the spirometer had emptied to one-third its capacity. Oxygen was then admitted until the spirometer assumed its initial position. With this procedure the oxygen in the tank was never reduced to a point interfering with the normal loading of oxygen by the blood in the lungs. The oxygen was reduced to not less than 18 per cent.

It is obvious that a change in temperature of the gas within the rebreathing apparatus during any single observation would interfere with the accuracy of the method of measuring the rate of oxygen consumption. A rise in temperature would retard the descent of the spirometer and give low values, and a fall in temperature would accelerate the descent of the spirometer and give high values.

We have followed the temperature of the air in rebreathing apparatus in several experiments and though we have noted very slow changes in temperature covering long intervals of time we believe that these changes are too slow and too small to materially effect our observations which last as a rule only 2 minutes. Nevertheless, we wish to discuss very briefly the possible sources of temperature effects so that they may be analyzed in the discussion of some of the data below.

In the course of these rebreathing experiments the air oscillates in a closed system between the lungs and the rebreathing apparatus. Since no effort is made to keep the rebreathing apparatus at the body temperature, the blood circulating through the lungs may be looked upon

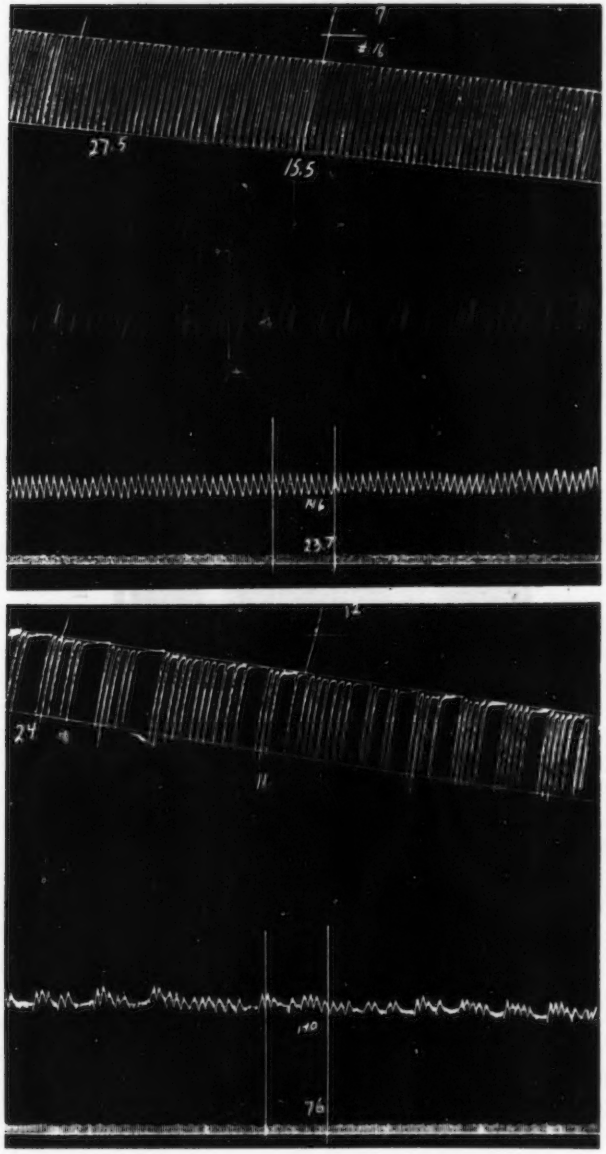

Fig. 1

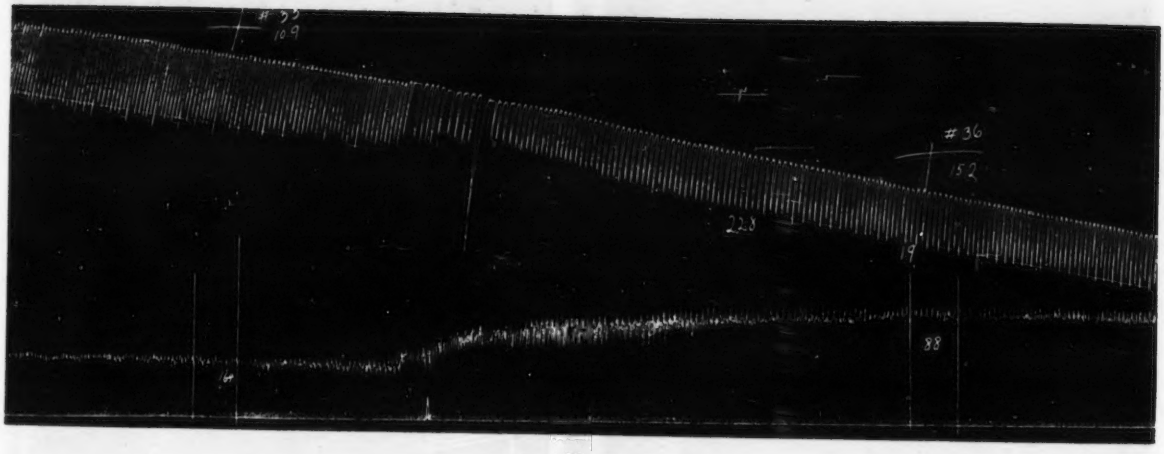

Fig. 2

as a source of heat to the air which is being rebreathed. A sudden change in either the rate of the circulation or in the body temperature of the dog, therefore, might alter the temperature of the air in the apparatus. Hemorrhage, by the sudden slowing of the circulation, theoretically might lead to a cooling of the respired air and increase the values for oxygen consumption. Injection of gum-saline solution would then produce the opposite results. If temperature effects from this source predominate over actual changes in metabolism, increased oxygen consumption should be indicated after hemorrhage and decreased consumption after the intravenous injection of gum-saline solution. The reverse, however, is almost invariably the result obtained.

If on the other hand the temperature of the air in the rebreathing apparatus is affected by the volume of air coming in contact with the blood, the descent of the spirometer would be retarded by increased ventilation and accelerated by decreased ventilation. Analysis of the tables will show that hemorrhage may increase, decrease or have no effect on the pulmonary ventilation. The same holds for the injection of gum-saline solution. With relatively few exceptions, however, as pointed out above, the results have shown a remarkable consistency. We have been unable to link our results, as will be apparent later on, with changes in pulmonary ventilation.

It should be pointed out, however, that we have not checked the graphic method against the analytical method and therefore can make no statement as to the absolute accuracy of our results. As a matter of fact, we believe the results are sometimes affected by slight changes in the expiratory point of rest occasioned by irregular breathing. Such breathing may possibly account for irregularities in results which will be pointed out from time to time. We have reason to believe, nevertheless, that on the whole fairly accurate data were obtained: e.g., the fact that absolutely straight gradients of the line of expiratory rest may frequently be obtained over periods of 15 minutes or more; also reduced rates of oxygen consumption may be recorded over periods of an hour or more, and subsequent to injection, long periods of increased oxygen may be recorded. In such observations, errors due to shifting of the line of expiratory rest are eliminated. The exact procedure of experimentation will be observed from the examination of the tables of each experiment.

Results. Eight experiments were performed. The data are tabulated and discussed in the order in which the experiments occurred. The

tables show from left to right, the number of the observation, the oxygen consumption, in cubic centimeters per kilogram of body weight per minute, the respiratory and pulse rate per minute, the mean blood pressure and solar time. Hemorrhages and injections are noted in cubic centimeters and in percent of body weight. Percentage of hemoglobin is also noted from time to time.

Experiment 1. (*See table 1.*) Hemorrhage 1, amounting to 1.65 per cent of the body weight, reduced the total oxygen consumption from an average of 5.56 cc. to 4.84 cc. per kilogram of body weight per minute, a reduction of 13 per cent. A second hemorrhage amounting to 0.50 per cent of the body weight reduced the consumption of oxygen still more. Injection 1, following hemorrhage 2, markedly increased the oxygen consumption to an average value of 5.56 cc. per kilogram of body weight per minute (see observations 7, 8 and 9). The third hemorrhage of 1.02 per cent of the body weight though again reducing the oxygen consumption did not lower it as much as the first hemorrhage which was considerably larger. The second injection had the same augmenting effect on the oxygen consumption as the first injection (see observations 15, 16 and 17). The next four successive hemorrhages, nos. 4, 5, 6 and 7, produced striking decreases in the rate of oxygen consumption. During observation 21, following hemorrhage 7, the consumption was only 30 per cent of that following injection 2. The last injection elicited an average increase in the oxygen consumption amounting to 261 per cent (see observations 22, 23 and 24).

Although in general the fluctuations of oxygen consumption followed the fluctuations in blood volume, there were some exceptions. Fluctuations occurred when the volume of the blood presumably remained the same; e.g., observations 5 and 6 following hemorrhage 2; observations 7, 8, 9, 10 and 11 following injection 1; observations 15, 16 and 17 following injection 2; and observations 22, 23 and 24 following injection 3. Some of these fluctuations appear to be connected with the magnitude of mean blood pressure; e.g., observations 5 and 6. The higher rate of oxygen consumption was associated with the higher mean blood pressure. As a matter of fact the fluctuations in oxygen consumption followed the mean blood pressure very closely in this experiment which perhaps is to be expected in so far as changes in blood-volume invariably produced large changes in mean blood pressure. Yet later experiments in which changes in blood-volume occurred without any or with small changes in mean blood pressure show that oxygen consumption as a rule is more closely dependent on blood-volume than it is on mean blood pressure.

TABLE 1

Experiment 1

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	5.60	228	26	182	132	11.55
2	5.65	221	26	164	126	
3	5.42	219	25	152	128	
Blood sample 1. Hemoglobin 136 per cent						
Hemorrhage 1—162 cc.—1.65 per cent body weight						
4	4.84	239	25	154	92	12.24
Hemorrhage 2—49 cc.—0.50 per cent body weight						
5	4.15	251	28	130	58	
6	4.44	236	25	144	68	
Blood sample 2. Hemoglobin 135 per cent						
Injection 1—230 cc.—235 per cent body weight						
7	6.34	295	27	192	76	12.48
8	5.54	281	28	154	99	
9	5.13	274	33	162	80	
Blood sample 3. Hemoglobin 108 per cent						
10	5.02	360	39	170	82	1.05
11	5.77	333	37	182	90	
Hemorrhage 3—100 cc.—1.02 per cent body weight						
12	5.02	380	38	178	50	1.25
13	5.12	310	31	172	57	
14	5.12	307	36	176	62	1.33
Blood sample 4. Hemoglobin 104 per cent						
Injection 2—160 cc.—1.63 per cent body weight						
15	7.21	337	34	176	98	1.41
16	6.34	350	38	176	106	
17	6.75	341	37	172	110	
Blood sample 5. Hemoglobin 91 per cent						
Hemorrhage 4—56 cc.—0.57 per cent body weight						
18	6.34	405	37		96	1.51
Hemorrhage 5—42 cc.—0.43 per cent body weight						
19	5.65	460	46	190	75	1.57
Hemorrhage 6—51 cc.—0.52 per cent body weight						
20	4.32	407	43	192	57	2.05
Hemorrhage 7—46 cc.—0.47 per cent body weight						
21	2.36	285	25	176	23	2.10
Injection 3—250 cc.—2.55 per cent body weight						
22	7.26	415	30	150	68	
23	5.59	385	38	160	81	
24	6.10	392	41	172	88	2.26
Blood sample 6. Hemoglobin 43 per cent						

Although injections 1, 2 and 3 increased the rate of oxygen consumption and the mean blood pressure yet the respective sets of observations, 7, 8 and 9; 15, 16 and 17; 22, 23 and 24 might be looked upon as exceptions to both generalizations that oxygen consumption varies with the blood-volume and with the mean blood pressure. Presumably the blood-volume remained constant during each particular set of observations yet it will be noted that the first observation of each set shows decidedly the highest rate of oxygen consumption. It will also be noted that the highest rate of oxygen consumption occurred during the lowest mean blood pressure. The high rate of oxygen consumption at the beginning of each observation is probably due to oxygen hunger following the antecedent periods of "anoxemia." Doi (4) who obtained comparable results on the cat, also offers this suggestion. The fact that the blood pressure does not mount abruptly to its maximum after injection, but slowly, may support this view in that it suggests a gradual recovery of the tissues following the improvement in the supply of oxygen and other nutrients.

It might be well to analyze the results on oxygen consumption in relation to possible changes in temperature of the air in the rebreathing apparatus associated with changes in the volume-flow of blood through the lungs, and also with the minute volume of pulmonary ventilation. If a decrease in the volume-flow of blood leads to a prompt cooling of the air and accelerates the descent of the spirometer and if this is the only disturbing factor, the effects of changes in blood-volume were greater than those recorded. If, on the other hand, increased pulmonary ventilation leads to a prompt warming of the air and retards the descent of the spirometer, there are many instances in which this factor might produce apparent reductions in the amount of oxygen consumed after hemorrhage. Examples of such instances are seen in observations 18 and 19 after hemorrhages 4 and 5 respectively in which each hemorrhage was followed by a large increase in the pulmonary ventilation. The subsequent hemorrhages 6 and 7, however, produced large reductions in the pulmonary ventilation and undoubtedly striking reductions in the volume-flow of blood, yet slower rates of oxygen consumption continued to be recorded. Just as significant is observation 22 following injection 3 which increased the pulmonary ventilation enormously and undoubtedly increased the volume-flow of blood. Despite these two factors, each of which theoretically might retard the descent of the spirometer, the records indicated an increased rate of oxygen consumption.

(Further analysis of the tables in relation to temperature changes in the air in the rebreathing apparatus will not be made in the following experiments.)

Experiment 2. (*See table 2.*) In this experiment in which the effects of nine hemorrhages and five injections were studied, each hemorrhage was followed by a decrease in the rate of oxygen consumption and each injection of gum-saline solution was followed by an increase in the rate of oxygen consumption. The results are comparable to those obtained in experiment 1. There is a significant point of difference, however, occurring at the beginning of these experiments. It will be recalled that the hemorrhages employed in experiment 1 invariably produced a decided fall in the mean blood pressure. In experiment 2 the initial hemorrhage, which was only 0.50 per cent of the body weight, had little effect upon the mean blood pressure. It reduced it only 4 mm. of mercury and yet decreased the rate of oxygen consumption 15 per cent. Such observations indicate that hemorrhages eliciting very small changes in the mean blood pressure may be accompanied by circulatory disturbances of sufficient gravity to interfere with the normal rate of oxygen consumption.

Note again the hyper-oxygen consumption occurring immediately after the injection of gum-saline solution. Compare the values of oxygen consumption in observations 9 and 10 after injection 2; in observations 13 and 14 after injection 3; and observations 19 and 20 after injection 5.

We call attention to the marked effects of hemorrhages 7 and 8 in observations 15 and 16. Accepting the lower figure 4.97 cc. (observation 14) as the basal value of oxygen consumption in the period following injection 3, we find that the rate of oxygen consumption was reduced 27 per cent by these two hemorrhages.

If we accept 4.46 cc. per kilogram of body weight per minute as representing the basal oxygen consumption after injection 5 (observation 20) and take the average oxygen consumption of observations 1, 2 and 3 as representing the initial rate of oxygen consumption, we note a total reduction in the oxygen consumption amounting to 37 per cent. But coupled with this reduction there was a fall in the percent of hemoglobin from 120 per cent to 31 per cent, a total reduction of 74 per cent. The results indicate that either the "coefficient of utilization of oxygen" or the volume-flow of blood was enormously increased. It is quite probable that both were increased. We made no direct determinations of the blood-volume, but knowing the amounts bled and injected,

TABLE 2

Experiment 2

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	7.30	263	16	152	120	10:07
2	7.77	263	16	166	120	
3	7.36	265	16	184	120	
Blood sample 1. Hemoglobin 120 per cent						10.15
Hemorrhage 1—53 cc.—0.50 per cent body weight						
4	6.21	244	15	160	116	
Hemorrhage 2—56 cc.—0.52 per cent body weight						
5	5.30	230	15	156	98	10.32
Blood sample 2. Hemoglobin 116 per cent						
Hemorrhage 3—51 cc.—0.48 per cent body weight						
6	4.98	199	15	168	79	
Blood sample 3. Hemoglobin 109 per cent						
Hemorrhage 4—53 cc.—0.50 per cent body weight						
7	4.15	214	16	168	60	10.41
Blood sample 4. Hemoglobin 105 per cent						
Injection 1—125 cc.—1.17 per cent body weight						
8	5.95	237	15	200	85	10.51
Blood sample 5. Hemoglobin 87 per cent						11.05
Injection 2—125 cc.—1.17 per cent body weight						
9	6.48	286	19	200	112	
10	6.32		19	196	103	
Blood sample 6. Hemoglobin 83 per cent						11.33
Hemorrhage 5—57 cc.—0.53 per cent body weight						
11	5.08	290	20	180	64	
Hemorrhage 6—51 cc.—0.48 per cent body weight						
12	4.10			160	42	
Blood sample 7. Hemoglobin 73 per cent						
Injection 3—125 cc.—1.17 per cent body weight						11.58
Blood sample 8. Hemoglobin 65 per cent						
13	6.59	351	26	184	77	
14	4.97	328	24	184	74	12.10
Hemorrhage 7—50 cc.—0.47 per cent body weight						
15	4.71	300	20	181	52	12.19
Blood sample 9. Hemoglobin 58 per cent						
Hemorrhage 8—57 cc.—0.53 per cent body weight						
16	3.64	236	17	134	37	12.28
Blood sample 10. Hemoglobin 54 per cent						

TABLE 2—*Concluded*

Experiment 2—Concluded

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Injection 4—150 cc.—1.60 per cent body weight						
17	5.76	263	26	196	76	12.40
Blood sample 11. Hemoglobin 44 per cent						
Hemorrhage 9—43 cc.—0.44 per cent body weight						
18	2.95	258	16	162	40	
Injection 5—220 cc.—2.24 per cent body weight						
19	5.28	415	31	170	81	12.56
20	4.46	343	28	184	91	1.08
Blood sample 12. Hemoglobin 31 per cent						

we are safe in assuming that a definite hydremic plethora prevailed during observation 20. Undoubtedly this produced a volume-flow of blood greater than that at the beginning of the experiment. But since we have no direct data on the volume-flow of blood, no definite statements can be made concerning the coefficient of utilization of oxygen consumption. It should be remarked here that the temperature of the animal remained constant throughout the experiment.

Experiment 3. (*See table 3.*) The condition of this animal at the beginning of the experiment appeared very unstable as indicated by the fluctuations in the mean blood pressure before hemorrhage (observations 1 and 2) and after hemorrhage 1 (observations 3 and 4) and by the large fall in the mean blood pressure produced by a total hemorrhage of only 0.82 per cent of the body weight. On account of the critical condition of the animal a large injection of gum-saline was administered (observation 6) and time allowed for recovery. It will be noted that the injection was not very effective in elevating the pressure. Although the results up to observation 7 are not very regular they show in general the same effects of changes in blood-volume as noted in the preceding experiments.

After observation 6 the mean blood pressure, in the course of an hour, recovered to some extent. From this point on the data were more regular. Hemorrhage with only one exception decreased the amount of

TABLE 3

Experiment 3

OBSERVATION	OXYGENCONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	5.61	161	8	120	104	10.57
2	6.78	120	7	120	112	
Blood sample 1. Hemoglobin 100 per cent						11.21
Hemorrhage 1—44 cc.—0.35 per cent body weight						
3	5.91	141	10	120	109	
4	4.72	131	10	132	78	
Hemorrhage 2—60 cc.—0.47 per cent body weight						
5	4.82	192	12	130	45	11.36
Blood sample 2. Hemoglobin 104 per cent						
Injection 1—200 cc.—1.58 per cent body weight						
6	5.37	246	17	150	74	
Animal in bad condition. Intermission						
Injection 2—200 cc.—1.58 per cent body weight						12.37
Blood sample 3. Hemoglobin 91 per cent						
7	5.68	273	25	200	88	1.02
Blood sample 4. Hemoglobin 78 per cent						1.07
Hemorrhage 3—60 cc.—0.47 per cent body weight						
8	5.11	261	20	200	80	
Hemorrhage 4—60 cc.—0.47 per cent body weight						
9	4.93	348	24	192	74	1.13
10	4.20	257	19	164	76	
Blood sample 5. Hemoglobin 76 per cent						
Hemorrhage 5—62 cc.—0.49 per cent body weight						
11	4.28	327	22	158	62	
Hemorrhage 6—61 cc.—0.48 per cent body weight						
12	4.12	354	23	140	50	1.33
Blood sample 6. Hemoglobin 70 per cent						
Injection 3—280 cc.—2.21 per cent body weight						
13	5.11	365	25	155	106	1.47
Blood sample 7. Hemoglobin 57 per cent						
Hemorrhage 7—300 cc.—2.36 per cent body weight						
14	3.72	491	25	128	37	
Injection 4—400 cc.—3.15 per cent body weight						
15	4.59	514	39	150	94	2.10
16	5.61	562	30	176	116	
Blood sample 8. Hemoglobin 36 per cent						
Hemorrhage 8—261 cc.—2.06 per cent body weight						

TABLE 3—*Concluded*

Experiment 3—Concluded

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
17	4.59	614	27	156	45	2.33
Injection 5—400 cc.—3.15 per cent body weight						
18	5.46	679	36	140	90	
Blood sample 9. Hemoglobin 22 per cent						
Hemorrhage 9—205 cc.—1.61 per cent body weight						
19	3.93	549	23	128	56	
Injection 6—300 cc.—2.36 per cent body weight						
20	4.20	736	36	144	61	
Blood sample 10. Hemoglobin 16 per cent						3.20

oxygen consumed and injection invariably increased it. In observation 15 following injection 4 the rate of oxygen consumption was considerably increased over that obtaining during the preceding period of smaller blood-volume. In the following observation (no. 16) also after injection 4 the oxygen consumption increased still more. These results differ from those previously described in which a hyper-oxygen consumption occurs immediately after injection and gives way to a somewhat lower consumption. It is probable that the tissues suffered from the effects of hemorrhage 7. This hemorrhage elicited a very low mean blood pressure and an oxygen consumption of only 60 per cent of the initial rate. Such marked anoxemia particularly near the close of an experiment presumably may not always be recovered from with the promptness noted in the preceding experiments. Perhaps the prompt reaction of the hyper-oxygen consumption following injection can occur only in tissues which are in fairly good condition.

The reduction in the rate of oxygen consumption resulting from hemorrhage was considerable in this experiment, yet it was not comparable to the reduction in the percentage of hemoglobin which occurred. The absence of data on the volume-flow of blood prevents us from reaching conclusions concerning the coefficient of utilization of oxygen.

Experiment 4. (*See table 4.*) It has been our effort in this series of experiments to determine the effects of small hemorrhages which have little or no effect on mean blood pressure as well as of hemorrhages

TABLE 4

Experiment 4

OBSERVATION	OXYGENCONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	18.20	222	13	88	127	10.10
2	17.60	287	15	88	128	
Blood sample 1. Hemoglobin 122 per cent						
Hemorrhage 1—46 cc.—0.50 per cent body weight						
3	15.00	220	13	92	127	10.25
Hemorrhage 2—45 cc.—0.49 per cent of body weight						
4	12.70	218	14	76	112	10.37
Hemorrhage 3—45 cc.—0.49 per cent body weight						10.47
Blood sample 2. Hemoglobin 130 per cent						
5	12.50	229	14	78	70	
Injection 1—100 cc.—1.08 per cent body weight						
6	13.20	300	8	126	60	10.55
Blood sample 3. Hemoglobin 135 per cent						
Injection 2—50 cc.—0.54 per cent body weight						
7	14.40	316	11	140	76	11.07
Blood sample 4. Hemoglobin 137 per cent						
Injection 3—100 cc.—1.08 per cent body weight						
8	10.80	326	16	138	76	11.35
Blood sample 5. Hemoglobin 128 per cent						11.39
Injection 4—100 cc.—1.08 per cent body weight						
9	11.90	401	20	130	78	
Blood sample 6. Hemoglobin 116 per cent						
Hemorrhage 4—45 cc.—0.49 per cent body weight						11.50
10	11.50	371	18	150	54	
Hemorrhage 5—47 cc.—0.51 per cent body weight						
11	10.30	372	15	164	39	11.59
Blood sample 7. Hemoglobin 106 per cent						12.01
Injection 5—60 cc.—0.65 per cent body weight						
12	10.45	373	13	160	54	
Injection 6—60 cc.—0.65 per cent body weight						
13	10.92	348	12	142	72	12.16
Blood sample 8. Hemoglobin 90 per cent						
Hemorrhage 6—103 cc.—1.11 per cent body weight						
14	8.40	459	14	150	42	12.29
Blood sample 9. Hemoglobin 87 per cent						12.37
Injection 7—150 cc.—0.59 per cent body weight						
15	10.80	470	14	168	76	

TABLE 4—*Concluded*

Experiment 4—Concluded

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Blood sample 10. Hemoglobin 71 per cent						12.50
Hemorrhage 7—99 cc.—1.07 per cent body weight						
16	8.40	511	15	146	47	
Blood sample 11. Hemoglobin 64 per cent						1.00
Injection 8—130 cc.—1.41 per cent body weight						
17	10.55	529	16	144	70	
Blood sample 12. Hemoglobin 52 per cent						
Hemorrhage 8—178 cc.—1.92 per cent body weight						
18	7.08	526	19	94	34	
Injection 9—210 cc.—2.27 per cent body weight						
19	11.15	598	17	140	72	1.18

which produce marked reductions in mean blood pressure. Reductions in the rate of oxygen consumption resulting from hemorrhages having small effects on mean blood pressure were described in experiments 2 and 3. In observation 3 of experiment 4, we have an example of a large reduction in the rate of oxygen consumption produced by a hemorrhage which had no effect upon the mean blood pressure. Further hemorrhages reduced the oxygen consumption still more (see observation 4 and 5) and subsequent injections 1 and 2 decidedly increased the oxidations.

The initial rate of oxygen consumption in this experiment was exceedingly high compared with the consumption in other animals. Perhaps this accounts for the striking effects elicited by small hemorrhage. After injection 3 the rate of oxygen consumption fell off abruptly. It was increased again by injection 4 but never reached the high initial level; but later the effects of changes in blood-volume on oxygen consumption continued with perfect regularity.

Whether the sudden reduction in the rate of oxygen consumption was due to a toxic action of gum-acacia; to a sudden deterioration of the tissues from anoxemia; to an accidental closure of a leak in the apparatus or to some other cause, we cannot say. Leaks were tested for at the beginning of every experiment. But it might be pointed out that

the animal reacted to hemorrhage in a peculiar way. The first hemorrhage produced practically no change in either the pulse rate or the mean blood pressure. The second hemorrhage was followed by a striking reduction in the pulse rate which was maintained up through observation 5. Following observation 5 the pulse became irregular and more rapid resembling a pulse escaping from vagal block. Immediately before injecton 1, the pulse suddenly accelerated and subsequently the pulse rate showed the usual changes of slowing with injection and accelerating with hemorrhage.

Experiment 5. (*See table 5.*) The effects of fifteen hemorrhages and fifteen injections were noted in this experiment. As the tables indicate, the first two hemorrhages were followed by increased oxygen consumption, the third by decreased consumption, and the fourth by increased consumption. The results continued in this irregular fashion up to observation 14, after which they became perfectly regular. We have no definite explanation to offer other than those already offered in the discussion of the method, unless it is that these irregularities are due to circulatory compensations. Meek and Eyster (5) employing the x-ray shadow of the heart as an index to the cardiac output conclude that initial hemorrhages frequently exert no effect on the volume output. Aub (6) finds that hemorrhage does not always reduce the metabolism of the anesthetized cat. It is possible that the irregularity in results occurring at the beginning of this and other experiments stands in agreement with these findings.

It is interesting that the results, after becoming regular, should be so marked. Observe for example the values 8.26 cc., 6.12 cc., 8.65 cc. and 5.80 cc. oxygen consumption per kilogram of body weight occurring after alternate injections and hemorrhages in observations 23, 24, 25 and 26. These are relatively high values for oxygen consumption and they occurred toward the end of the experiment when the hemoglobin values had fallen considerably. During observation 25 when 8.65 cc. of oxygen per kilogram of body weight per minute were consumed there was only 22 per cent of hemoglobin. But as the hemoglobin values fell still lower, marked reductions in the oxygen consumption during periods in which the blood-volumes were approximately equal occurred. Compare the values of oxygen consumption after hemorrhages 12, 13, 14 and 15 and after injections 12, 13, 14 and 15. In observations 26, 28, 30 and 32 following hemorrhages 12, 13, 14 and 15 the oxygen consumption was reduced from 5.80 cc. to 5.56 cc. to 3.57 cc. to 2.38 cc. per kilogram of body weight per minute. These

TABLE 5

Experiment 5

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	6.70	303	26	120	114	
Blood sample 1. Hemoglobin 106 per cent						
Hemorrhage 1—34 cc.—0.49 per cent body weight						
2	6.95	326	28	114	114	10.19
Blood sample 2. Hemoglobin 100 per cent						
Hemorrhage 2—40 cc.—0.58 per cent body weight						
3	7.20	283	24	112	100	10.29
Blood sample 3. Hemoglobin 98 per cent						
Hemorrhage 3—34 cc.—0.49 per cent body weight						
4	5.67	272	21	126	93	10.42
Blood sample 4. Hemoglobin 100 per cent						
Hemorrhage 4—37 cc.—0.53 per cent body weight						
5	6.54	268	20	168	88	10.52
Blood sample 5. Hemoglobin 99 per cent						
Hemorrhage 5—35 cc.—0.53 per cent body weight						
6	6.20	291	22	190	88	
7	7.20	294	23	202	90	
Blood sample 6. Hemoglobin 92 per cent						
Hemorrhage 6—38 cc.—0.55 per cent body weight						
8	6.35	253	18	152	66	11.20
Blood sample 7. Hemoglobin 93 per cent						
Injection 1—40 cc.—0.57 per cent body weight						
9	6.82	343	24	202	72	11.32
Injection 2—40 cc.—0.57 per cent body weight						
10	6.51	413	28	190	80	11.42
Blood sample 8. Hemoglobin 75 per cent						
Injection 3—40 cc.—0.57 per cent body weight						
11	6.43	414	29	178	88	11.50
Injection 4—40 cc.—0.57 per cent body weight						
12	7.38	443	31	152	98	12.00
Blood sample 9. Hemoglobin 71 per cent						
Injection 5—40 cc.—0.57 per cent body weight						
13	8.42	385	25	168	98	12.10
Injection 6—40 cc.—0.57 per cent body weight						
14	8.42	370	24	148	106	12.20
Blood sample 10. Hemoglobin 61 per cent						
Hemorrhage 7—50 cc.—0.72 per cent body weight						

TABLE 5—*Continued*

Experiment 5—Continued

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
15	6.95	381	24	174	88	12.38
Blood sample 11. Hemoglobin 60 per cent						
Hemorrhage 8—51 cc.—0.73 per cent body weight						
16	6.12	354	23	172	55	12.48
Blood sample 12. Hemoglobin 55 per cent						
Injection 7—60 cc.—0.86 per cent body weight						
17	7.54	388	24	190	76	12.57
Blood sample 13. Hemoglobin 51 per cent						
Injection 8—70 cc.—1.01 per cent body weight						
18	7.70	477	30	176	82	
Blood sample 14. Hemoglobin 45 per cent						
Injection 9—80 cc.—1.15 per cent body weight						
19	7.38	462	30	176	82	
Blood sample 15. Hemoglobin 41 per cent						
Hemorrhage 9—79 cc.—1.14 per cent body weight						
20	6.70	429	27	150	56	1.29
Blood sample 16. Hemoglobin 39 per cent						
Injection 10—80 cc.—1.15 per cent body weight						
21	7.70	540	34	176	85	1.46
Blood sample 17. Hemoglobin 35 per cent						
Hemorrhage 10—100 cc.—1.44 per cent body weight						
22	7.54	461	29	180	65	1.58
Blood sample 18. Hemoglobin 32 per cent						
Injection 11—140 cc.—2.02 per cent body weight						
23	8.26	517	35	154	103	2.20
Blood sample 19. Hemoglobin 27 per cent						
Hemorrhage 11—101 cc.—1.45 per cent body weight						
24	6.12	427	28	190	76	2.35
Blood sample 20. Hemoglobin 25 per cent						
Injection 12—110 cc.—1.58 per cent body weight						
25	8.65	556	35	150	92	2.51
Blood sample 21. Hemoglobin 22 per cent						
Hemorrhage 12—100 cc.—1.44 per cent body weight						
26	5.80	417	26	152	56	3.05
Blood sample 22. Hemoglobin 20 per cent						
Injection 13—130 cc.—1.87 per cent body weight						
27	7.30	561	35	190	92	3.13

TABLE 5—*Concluded*

Experiment 5—Concluded

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
Blood sample 23. Hemoglobin 17 per cent						
Hemorrhage 13—102 cc.—1.47 per cent body weight						
28	5.56	481	27	140	58	3.29
Blood sample 24. Hemoglobin 17 per cent						
Injection 14—130 cc.—1.87 per cent body weight						
29	6.27	550	32	150	98	3.39
Blood sample 25. Hemoglobin 14 per cent						
Hemorrhage 14—126 cc.—1.81 per cent body weight						
30	3.57	532	25	144	73	3.57
Injection 15—150 cc.—2.16 per cent body weight						
31	5.56	679	25	120	79	4.11
Blood sample 26. Hemoglobin 10 per cent						
Hemorrhage 15—184 cc.—2.65 per cent body weight						
32	2.38	557	34	102	73	4.16
Blood sample 27. Hemoglobin 7 per cent						

reductions in oxygen consumption were accompanied by reductions in the percentage of hemoglobin from 20 per cent to 17 per cent to 14 per cent to 7 per cent.

Experiment 6. (*See table 6.*) The average rate of oxygen consumption for observations 1, 2 and 3 before hemorrhage was 13.58 cc. per kilogram of body weight per minute. Hemorrhage 1 produced an immediate reduction in the rate, the effects becoming progressively more marked up to observation 8, after which the oxygen consumption increased again. The hemoglobin value of blood sample 2 indicates an increase in blood-volume which may have led to the improved oxidations and prevented a further improvement from injection 1. The results after injection 1 up to observation 28 are rather irregular, possibly due in part to the incomplete absorption of carbon dioxide by the soda-lime. After renewal of soda-lime the values of oxygen consumption became greater and showed a regular and marked response to changes in blood-volume from observation 29 to 36, the end of the experiment. We call particular attention to the effect of hemorrhage 7 which reduced the oxygen consumption from 14.40 cc. to 7.94 cc. per

TABLE 6

Experiment 6

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	13.98	189	14	124	110	2.28
2	13.35	221	13	134	118	
3	13.40	252	14	138	127	
Blood sample 1. Hemoglobin 118 per cent						
Hemorrhage 1—82 cc.—0.52 per cent body weight						
4	12.78	270	14	172	122	3.04
5	12.43	270	14	166	120	
6	12.29	270	14	190	118	
7	11.87	287	14	220	115	
8	11.52	276	14	220	115	
9	12.15	286	14	240	114	
10	12.00	283	14	256	114	
11	12.36	315	15	244	115	
Blood sample 2. Hemoglobin 114 per cent						
Injection 1—85 cc.—0.54 per cent body weight						
14	12.00	320	16	180	114	3.37
16	12.50	358	16	186	114	
17	11.74	358	16	180	113	
18	11.93	362	16	180	114	
Blood sample 3. Hemoglobin 110 per cent						
Hemorrhage 2—85 cc.—0.54 per cent body weight						
21	10.88	377	16	220	106	3.50
22	12.43	393	16	220	106	
Blood sample 4. Hemoglobin 108 per cent						
Injection 2—85 cc.—0.54 per cent body weight						
23	11.23	417	17	154	114	4.01
Blood sample 5. Hemoglobin 104 per cent						
Hemorrhage 3—83 cc.—0.53 per cent body weight						
24	10.80	503	19	194	108	4.10
Injection 3—80 cc.—0.51 per cent body weight						
25	10.60	528	19	156		
Blood sample 6. Hemoglobin 97 per cent						
Hemorrhage 4—85 cc.—0.54 per cent body weight						
26	9.49	590	20	170	114	
Injection 4—85 cc.—0.54 per cent body weight						
27	9.49	670	22	147	121	
Soda lime renewed						

TABLE 6—*Concluded*

Experiment 6—Concluded

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
28	14.05	263	17	146	126	
Blood sample 7. Hemoglobin 88 per cent						
Hemorrhage 5—154 cc.—0.97 per cent body weight						
29	14.05	267	18	198	112	
Blood sample 8. Hemoglobin 86 per cent						
Injection 5—170 cc.—1.08 per cent body weight						
30	16.00	284	19	154	123	
Blood sample 9. Hemoglobin 75 per cent						
Hemorrhage 6—290 cc.—1.84 per cent body weight						
31	10.32	300	20	180	55	
Injection 6—330 cc.—2.09 per cent body weight						
32	14.40	328	25	156	108	
Blood sample 10. Hemoglobin 66 per cent						
Hemorrhage 7—258 cc.—1.63 per cent body weight						
33	7.94	545	28	186	44	
Blood sample 11. Hemoglobin 49 per cent						
Injection 7—280 cc.—1.77 per cent body weight						
34	12.86	398	28	196	97	
Blood sample 12. Hemoglobin 36 per cent						
Hemorrhage 8—247 cc.—1.56 per cent body weight						
35	7.66	498	35	164	43	
Injection 8—300 cc.—1.90 per cent body weight						
36	10.67	304	19	88	36	
Blood sample 13. Hemoglobin 23 per cent						

kilogram of body weight per minute. Note the large fall in blood pressure which accompanies this reduction in the oxygen consumption.

Experiment 7. (*See table 7.*) The results on the rate of oxygen consumption were irregular and contradictory up to observation 10, after which the usual effects of changes in blood-volume were obtained. Hemorrhages and injections amounting to about 0.50 per cent of the body weight elicited definite changes in the amount of oxygen consumed.

Experiment 8. (*See table 8.*) Experiment 8 was another example of irregular results at the beginning of an experiment, giving way to regular results as the experiment progressed. Regular results were obtained

TABLE 7

Experiment 7

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	7.00	377	27	252	82	12.05
Blood sample 1. Hemoglobin 126 per cent						
2	7.00	350	25	209	85	
3	6.00	342	25	177	85	
4	6.00	333	24	180	85	
5	6.28	336	24	195	85	
Blood sample 2. Hemoglobin 128 per cent						
Hemorrhage 1—47 cc.—0.48 per cent body weight						
6	7.16	360	24		67	12.37
7	6.18	360	24		78	
8	6.93	336	24		76	
9	6.28	362	25		74	
Blood sample 3. Hemoglobin 135 per cent						
Injection 1—50 cc.—0.52 per cent body weight						
10	7.16	357	25	172	91	12.58
11	7.04	356	26	167	94	
12	7.27	370	27	160	95	
13	6.87	360	25	156	96	
Blood sample 4. Hemoglobin 110 per cent						
Hemorrhage 2—50 cc.—0.52 per cent body weight						1.39
14	6.58	417	27	189	91	
Injection 2—50 cc.—0.52 per cent body weight						
15	7.03	405	27	182	96	1.47
Blood sample 5. Hemoglobin 104 per cent						
Hemorrhage 3—58 cc.—0.60 per cent body weight						
16	7.44	528	32	217	85	1.57
Blood sample 6. Hemoglobin 86 per cent						
Hemorrhage 4—54 cc.—0.56 per cent body weight						2.47
17	6.29	504	29	230	64	2.57
Injection 3—50 cc.—0.52 per cent body weight						
18	7.24	519	33	198	71	
Blood sample 7. Hemoglobin 82 per cent						
Hemorrhage 5—55 cc.—0.57 per cent body weight						
19	6.74	567	28		57	3.08
Injection 4—70 cc.—0.72 per cent body weight						
20	10.65	752	34		65	3.19
Blood sample 8. Hemoglobin 67 per cent						
Hemorrhage 6—99 cc.—1.02 per cent body weight						
21	6.70	557	22		39	3.37
Injection 5—120 cc.—1.24 per cent body weight						
22	7.55	575	26		56	
Blood sample 9. Hemoglobin 49 per cent						3.52

TABLE 8

Experiment 8

OBSERVATION	OXYGENCONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
1	7.60	471	33	194	94	4.09
2	8.00	435	32	182	97	
3	7.38	445	32	186	100	
4	7.21	380	27	182	103	
Blood sample 1. Hemoglobin 98 per cent						
Hemorrhage 1—35 cc.—0.49 per cent body weight						
5	8.91	423	33	170	100	4.29
6	9.85	423	33	176	102	
7	9.69	438	33	176	104	
8	9.15	373	31	176	106	
Blood sample 2. Hemoglobin 95 per cent						
Injection 1—40 cc.—0.56 per cent body weight						
9	8.60	422	31	160	114	4.50
10	8.91	390	30	142	116	
11	8.20	347	25	150	120	
Blood sample 3. Hemoglobin 91 per cent						
Hemorrhage 2—36 cc.—0.51 per cent body weight						
12	8.12	371	32	152	119	5.13
Injection 2—40 cc.—0.56 per cent body weight						
13	8.60	413	30	144	119	
Period of dyspnea lasting about 25 minutes						
14	8.91	797	85	196	128	5.50
15	845	758	85	196	128	
Blood sample 4. Hemoglobin 87 per cent						
Hemorrhage 3—36 cc.—0.51 per cent body weight						
16	837	824	85	202	122	
Injection 3—40 cc.—0.56 per cent body weight						
17	8.60	770	83	206	132	6.10
Blood sample 5. Hemoglobin 76 per cent						
Hemorrhage 4—35 cc.—0.49 per cent body weight						
18	8.20	780	86	204	123	
Injection 4—40 cc.—0.56 per cent body weight						
19	9.07	907	84	198	136	6.30
Blood sample 6. Hemoglobin 69 per cent						
Hemorrhage 5—36 cc.—0.51 per cent body weight						
20	8.05	976	96	180	110	7.00
Injection 5—40 cc.—0.56 per cent body weight						

TABLE 8—*Concluded*

Experiment 8—Concluded

OBSERVATION	OXYGEN CONSUMPTION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	VENTILATION IN CC. PER KGM. OF BODY WEIGHT PER MINUTE	RESPIRATORY RATE PER MINUTE	PULSE RATE PER MINUTE	MEAN BLOOD PRESSURE IN MM. Hg	TIME
21	8.67	856	75	220	116	
Blood sample 7. Hemoglobin 61 per cent						
Hemorrhage 6—38 cc.—0.54 per cent body weight						
22	6.88	606	51	200	75	
Injection 6—40 cc.—0.56 per cent body weight						
23	7.11	528	45	144	98	
Blood sample 8. Hemoglobin 55 per cent						
Hemorrhage 7—38 cc.—0.54 per cent body weight						
24	6.10	492	35	148	64	7.35
Injection 7—40 cc.—0.56 per cent body weight						
25	6.96	514	36	172	91	
26	6.80	523	38	200	91	
27	6.41	487	35	186	85	
Blood sample 9. Hemoglobin 49 per cent						
Hemorrhage 8—78 cc.—1.10 per cent body weight						
28	5.08	891	38	140	42	
29	6.10	682	35	132	42	
30	5.40	660	34	160	40	
Blood sample 10. Hemoglobin 47 per cent						
Injection 8—100 cc.—1.41 per cent body weight						
31	6.26	590	35	172	70	
32	7.19	531	35	166	77	
33	7.82	553	35	190	76	
Blood sample 11. Hemoglobin 38 per cent						

after observation 10 which occurred about one hour after the beginning of the experiment. The data of this experiment are significant in showing again that relatively small hemorrhages which have little effect on the mean blood pressure may elicit changes in the rate of oxygen consumption.

SUMMARY AND CONCLUSIONS

The relation of blood-volume to the rate of oxygen consumption was studied on the anesthetized dog.

The blood-volume was altered by hemorrhage and by the injection of gum-saline solution. The rate of oxygen consumption was studied by

means of a graphic method. The Henderson rebreathing apparatus was used which served to absorb the carbon dioxide of the expired air and record the rate at which the oxygen in the air was consumed.

Employing this method we found that the rate of oxygen consumption was markedly affected by changes in the blood-volume

With but few exceptions, hemorrhage decreased the rate of oxygen consumption and subsequent injection of gum-saline solution increased it.

In experiments in which hemorrhage was progressively increased, it was found in general that the greater the hemorrhage the slower the rate of oxygen consumption.

Small hemorrhages amounting to only 0.50 per cent of the body weight reduced the rate of oxygen consumption even though such hemorrhages had little or no effects upon the mean blood pressure. Such results show that a change in the rate of oxygen consumption resulting from a change in blood-volume is not necessarily dependent upon fluctuations in the mean blood pressure.

In one instance (exper. 6) a hemorrhage amounting to 1.63 per cent of the body weight reduced the rate of oxygen consumption from 14.40 cc. to 7.94 cc. per kilogram of body weight per minute. Subsequent injection of gum-saline solution amounting to 1.77 per cent of the body weight increased the oxygen consumption to 12.86 cc. per kilogram of body weight per minute. The mean blood pressures in these three observations were 108 mm. Hg., 44 mm. Hg. and 97 mm. Hg.

In another instance (exper. 4) a hemorrhage amounting to 0.50 per cent of the body weight reduced the oxygen consumption from 17.90 cc. to 15.00 cc. per kilogram of body weight per minute. The mean blood pressure remained the same—127 mm. during both observations.

By means of anesthesia we believe that we have eliminated the effects of changes of blood-volume upon muscular tonus. Although it is conceivable that hemorrhage and injection may influence the rate of oxygen consumption in several ways, we are inclined to ascribe the reduction and the increase in the consumption of oxygen consequent to hemorrhage and injection to changes in the volume-flow of blood.

The exceptions to the rule that hemorrhage decreases the rate of oxygen consumption occurring when the blood-volume is nearly normal, may indicate circulatory compensation involving the control of the venous supply of blood to the heart.

In experiments in which several observations on the rate of oxygen consumption were made following the injection of gum-saline solution,

the first observation, in most cases, showed a hyper-oxygen consumption which gave way in a few minutes to a lower rate of consumption. This lower rate of oxygen consumption, however, was considerably greater than that obtaining during the period of decreased blood-volume immediately before the injection. In some few cases, the initial rate of oxygen consumption following injection, though higher than that of the preceding period, increased still more.

These observations, we believe, are important in that they indicate that the decreased rate of oxygen consumption following hemorrhage is linked with a decreased supply of oxygen to the tissues. Reasons are given for ascribing the difference in the response to injection following a period of anoxemia to a difference in the condition of the tissues at the time of injection. The initial hyper-oxygen consumption probably occurs only when the tissues are in relatively good condition and are able to carry on rapid oxidations. The initial hypo-oxygen consumption probably occurs when the tissues are in a deteriorated condition and unable to utilize oxygen in a normal fashion. The increase in the rate of consumption following the initial hypo-oxygen consumption is probably due to an improvement of the condition of the tissues.

Since the gum-saline solution used in these experiments is in the ordinary sense a non-nutrient solution, the increased oxidations resulting from its injection have been ascribed to an increased nutrient-flow. Though the changes in oxygen consumption occurring with changes in blood-volume are probably primarily due to changes in the transport of oxygen, yet it is not improbable that the changes in the transport of other constituents of the blood may be of significance. But we call attention again to changes in the capillary blood pressure accompanying fluctuation in blood-volume. The consequent effects on the exchange of tissue fluids may also play a part in the alteration in the rate of oxygen consumption.

Since oxyhemoglobin becomes less acid when its oxygen is liberated and since hemorrhage almost invariably appears to decrease the oxygen consumption, the suggestion in the preceding paper that hemorrhage increases the respiratory response to the administration of a carbon dioxide mixture in room air, not only by a diminution of the transport of the carbon dioxide carriers but by a diminution of the transport of oxygen as well, is supported. Lack of oxygen may, therefore, act as a respiratory stimulus by virtue of the consequent diminution of the buffer action of the blood; i.e., lack of oxygen may excite the respiratory center indirectly by increasing the stimulating effect of the acid metabolites added to the blood.

We suggest that this and the increased or decreased effectiveness of acid metabolites liberated within the respiratory center itself in altering the hydrogen ion concentration of the center, due to synchronous changes in the extent of reduction of the oxyhemoglobin passing through the center, are of fundamental importance in the chemical control of respiration.

We believe that the dual function of hemoglobin offers a basis of a satisfactory explanation of various respiratory phenomena among which are included—the mode of action of lack of oxygen (low alveolar oxygen tension, carbon monoxide poisoning and cyanide poisoning), acapnia, periodic breathing, normal respiration, oxygen-poisoning and the mechanism of the stimulating effect of carbon dioxide and other acid metabolites. The details of these proposed explanations will follow in another paper.

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STUDIES OF GRAVES' SYNDROME AND THE INVOLUNTARY NERVOUS SYSTEM[1]

IV. The Vascular Response of the Pithed Cat to Single Intravenous Injections of Adrenalin

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Technique. The technical difficulties of measuring directly the activity of the involuntary nervous system are at present insurmountable. Indirect measurements are also unsatisfactory. We have taken for our indicator of the activity of the thoracico-lumbar division of the involuntary nervous system the vascular response of the pithed decerebrate cat to the intravenous injection of adrenalin. The site of action of adrenalin is the myoneural junction of the thoracico-lumbar system. Changes in the ganglia, in the post- and pre-ganglionic fibers, in the conduction of the nerve impulse, in the effector tissue cannot be evaluated. Despite these shortcomings this cat preparation is of considerable utility.

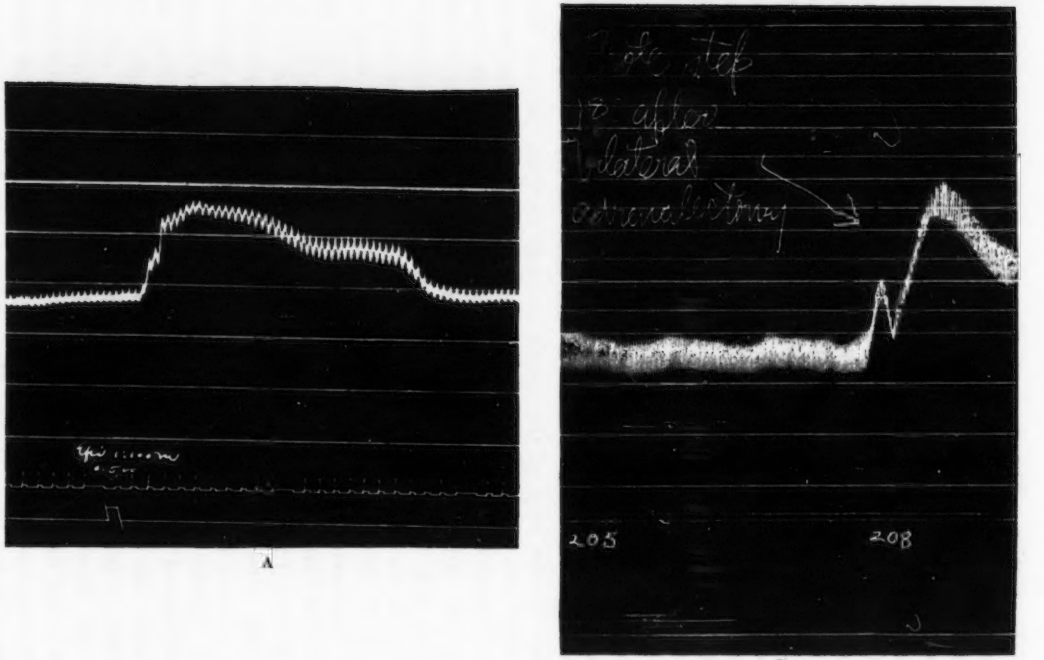
Fasting cats were weighed and etherized. The carotids were ligated and the trachea cannulized for artificial respiration;[2] the skull was trephined and the brain destroyed with a blunt spatula. The anesthetic was then discontinued and artificial respiration instituted. The cord was completely pithed by running a wire down the spinal canal; the blood pressure was recorded from the carotid of one side. The saphenous vein was prepared for the intravenous injection of adrenalin, which was run in from a burette graduated in tenths of a cubic centimeter. The adrenalin (Parke, Davis & Co.) was prepared fresh just before use, and diluted with distilled water or physiological saline to make a

[1] Papers I and II of this series will appear in the Amer. Journ. Med. Sci., paper III in Arch. Int. Med. early in 1923, and papers VIII and IX have appeared in Journ. Amer. Med. Assoc., 1922, lxxix, 1099; 1213. The Clinical Studies were made by Drs. Leo Kessel and H. T. Hyman.

[2] All apparatus employed was supplied by Joseph Becker, 437 West 59th Street, New York City.

1:100,000 solution. The amount of each injection, except where otherwise indicated, was 0.5 cc. The temperature of the animal was maintained by hot pads, by the heat of a drop light and by warming the inspired air. Aeration was mechanically maintained at a constant. This technique has been employed previously by Cushny (1), Elliott (2), Levy (3), Schultz (4), Barger and Dale (5), Houghton (6), Reid Hunt (7), Hoskins and Wheelon (8), Hoskins and Gunning (9), Wheelon and Shipley (10), Beifeld, Wheelon and Lavolet (11) and Hoskins and Rowley (12). The various minor differences in the protocols of previously reported experiments represent variables which we tried to avoid. The anesthesia, both the type and the depth, alters response (13). We eliminated this objection by stopping the anesthetic immediately after the central nervous system had been destroyed and permitting at least 30 minutes to elapse before beginning the experiment proper. Injections were made therefore in anesthetic-free animals. Pithing was carried beyond the fourth thoracic vertebra, insuring the complete destruction of the cord. Elliott (2) and Levy (3) have preferred to pith only to the level of the fourth thoracic vertebra, because they found that with complete pithing "peripheral dilatation of the vessels is often so great that the heart ceases to beat, and in any case the blood pressure rise caused by adrenalin becomes slow and irregular." We have been able to confirm the death of the animals from peripheral dilatation in the summer, so that it was necessary to abandon our experiments in the warm weather. In the winter these deaths were relatively infrequent, and usually occurred immediately after pithing. We have not found the blood pressure rise in completely pithed animals to be slow and irregular. With incomplete pithing, muscular movements may alter the blood pressure, and the blood pressure level is not as uniform as with complete destruction of the central nervous system. Where the pithing was complete the blood pressure tracing was maintained for hours at an absolute level. After pithing, the preparation was allowed to rest for from 15 to 60 minutes before the experiment proper was begun. All injections were made at a uniform rate. We have considered pithing and decerebration incomplete, if *1*, there is any movement of the voluntary musculature; *2*, if the blood pressure varies apparently spontaneously 15 per cent; *3*, if the blood pressure is higher than 50 mm. Hg., and we regarded with suspicion a pressure of over 40.

ANALYSIS OF THE VASCULAR RESPONSE TO SINGLE INTRAVENOUS INJECTIONS OF ADRENALIN. The effects of the intravenous injection of 0.5 cc. of 1:100,000 adrenalin may be described in four stages:



A B

Fig. 1. Illustrating persistence of "step" in adrenalin reaction following bilateral adrenalectomy. Complete response to adrenalin due to adrenalin injected. In B thyroidectomy also had been performed.

1. Latent period. In a fresh preparation there is always a short interval between the moment of injection and the response. In "tired" preparations, that is, after the elapse of several hours, this latent period is increased from 200 to 300 per cent.

2. The ascending limb. The rise is usually steep and may be uninterrupted or may show the "step" described by Elliott (2) and others. Their explanation of the mechanism of the step follows: that part of the rise from the baseline to the "step" is the direct vasconstrictor effect of the adrenalin injected intravenously; that part of the rise from the "step" to the fastigium is due to the outpouring of epinephrin,[3] secondary to excitation of the adrenal glands by adrenalin. To reinvestigate the mechanism of this "step," we have injected adrenalin into many pithed animals after ligation and extirpation of the adrenal glands. The removal of the adrenals in no way modifies this "step" (fig. 1). There seems little doubt, therefore, that splanchnic stimulation and the resultant outpouring of autogenous epinephrin plays no important rôle in the pressor response to injected adrenalin. The entire pressor response may be fairly attributed to the adrenalin injected. The only other possible factor to be considered is the accessory medullary tissue, the influence of which is probably negligible.

3. The fastigium. The fastigium in fresh preparations is usually short; in "tired" preparations it often assumes the plateau form.

4. The descent. The descent in the fresh preparation is usually abrupt, so that, with the doses which we employed the blood pressure returns to the original level within three or four minutes. In "tired" preparations there is a gentle slope rather than a steep descent, and the blood pressure does not return to its original level for seven or eight minutes. The prolonged latent period, the plateau type of fastigium, the gentle descent, and the prolonged duration of the response, have been taken as an indication of exhaustion of the animal. With these alterations in the response, the rise in the blood pressure is not diminished; in fact, it is usually increased. This observation is of importance in the interpretation of sensitiveness to adrenalin in human subjects, for, whereas sensitiveness is ordinarily interpreted as indicating increased tonicity (sympathicotony) our work would rather indicate an atony. Where there is myocardial exhaustion the vascular response is lessened both in height and duration. Adrenalin frequently produces extrasystoles and at times fibrillation.

[3] By epinephrin is meant the secretion of the adrenal medulla of the subject of the experiment. By adrenalin is meant the commercial preparation of Parke, Davis & Co.

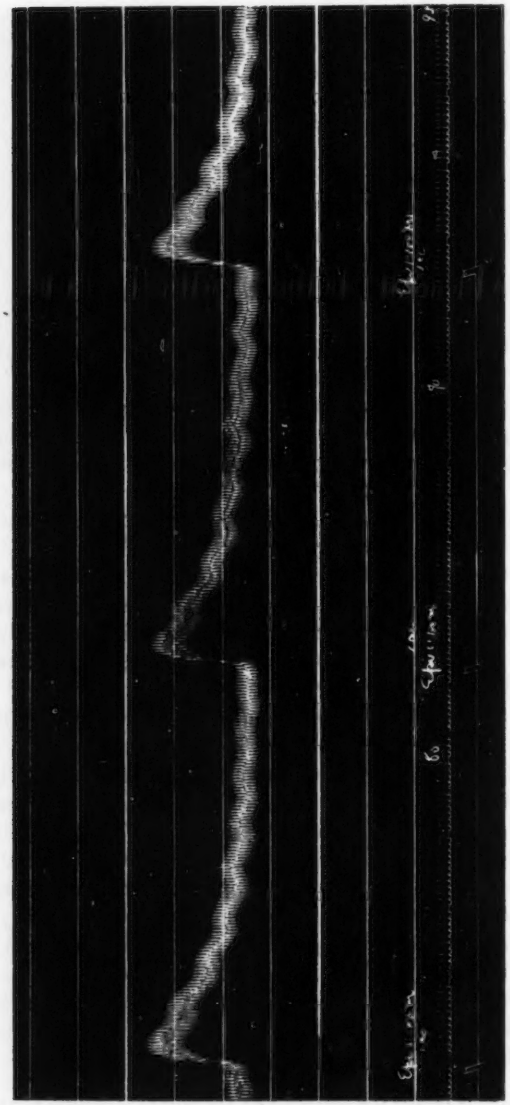


Fig. 2. Illustrating "reaction picture." Note intrinsic waves on blood pressure tracing. Each adrenalin response exactly duplicates the other.

The reaction is also characterized (14) by the appearance of small intrinsic waves on the descending limb of the curve, which persist after the blood pressure returns to normal (fig. 2). These waves are remarkably uniform and are so accurately reproduced that the curves may be superimposed. Usually these waves do not appear until after one or more injections of adrenalin. They persist after nicotine has paralyzed the ganglia and are also seen in isolated arteries; they are therefore peripheral in origin (fig. 3). We have come to think of them as an alternation of contraction and relaxation of the vessel wall, something

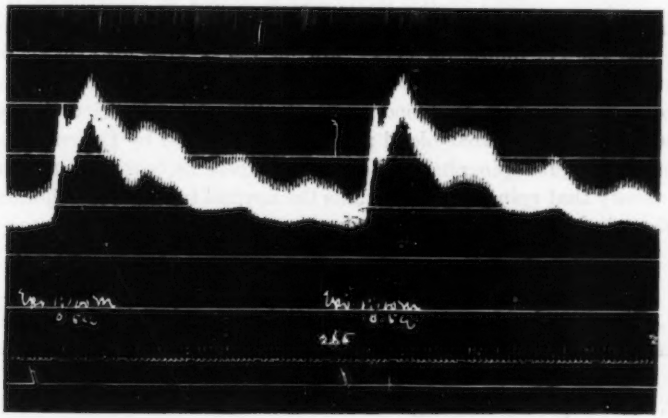


Fig. 3. Illustrating that intrinsic waves of "reaction picture" are independent of cord. Waves persist for 135 minutes after ligation of vertebral arteries and complete transection of cord in cervical region. Both sciatic nerves, previously isolated, cut and central ends stimulated with electrical current without alteration in blood pressure.

like the strife for equilibrium seen in the forearm when all the muscles are voluntarily tensed.

Minute doses of adrenalin cause at times not a rise but a fall in blood pressure—that is, a reversal of the usual response (15). This, unlike the pressor response, persists after the use of ergotoxine. The significance of this reversal phenomenon must be kept in mind. In the past, much confusion and fallacious reasoning have resulted from adhering to the conception that stimulation of the thoracico-lumbar nervous system invariably causes the same type of reaction.

The selective action of ergotoxine in paralyzing motor but leaving the inhibitory myoneural junctions intact indicates that the thoracico-lumbar system conveys protagonist as well as antagonist impulses. The result of its stimulation is the algebraic sum of a positive and a negative influence. Very small doses of adrenalin cause a dilatation of the blood vessels by stimulation of the dilator (inhibitory) nerves. Concentrations sufficient to cause complete inhibition of the intestinal musculature relax the blood vessels and cause a fall in blood pressure. Hoskins (16) rightly urges these facts as damaging evidence against the "tonicity" theory of the physiological action of epinephrin; for if epinephrin normally maintains vascular tonus, it must also hold the intestine in a state of inhibition (17). Further evidence against the tonicity theory is the observation that in acute experiments bilateral suprarenalectomy has no effect on blood pressure (18). The tonicity theory is opposed by the fact that blood pressure is unaltered by the continuous infusion of dilute epinephrin solution. All these facts render untenable the theory that epinephrin maintains the normal tonus of the thoracico-lumbar division of the involuntary nervous system.

CONCLUSIONS

1. The vascular responses of the pithed cat to epinephrin furnish a satisfactory index of the functional state of the myoneural junctions of the involuntary nervous system.

2. The response to a single intravenous injection is analyzed and its latent period, its ascending and descending limbs, and its fastigium are discussed.

3. When the animal is fatigued and the general reserve diminished, the period of latency becomes longer, the fastigium assumes a plateau type, the descending limb is gentler in its decline, and the extent of the rise in blood pressure is increased. In other words, the reaction is of longer duration and the restoration to normal is delayed. This indicates that sensitiveness to adrenalin accompanies atony, rather than increased tonicity, of the thoracico-lumbar division.

4. The entire response is due to the adrenalin injected. There is no evidence that adrenalin injected intravenously stimulates the adrenal glands and causes a secretion of epinephrin.

5. The response to a sympathomimetic stimulant drug is the sum of a positive and a negative influence. According to conditions, stimulation of the same nerve may cause qualitative as well as quantitative differences.

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STUDIES OF GRAVES' SYNDROME AND THE INVOLUNTARY NERVOUS SYSTEM

V. The Vascular Responses of the Pithed Cat to Repeated Intravenous Injections of Equal Doses of Adrenalin

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The vascular response of the pithed cat to repeated injections of equal doses of adrenalin was used as the indicator of the functional state of the peripheral components of the thoracico-lumbar division of the involuntary nervous system (1). In every delicate method of investigation, technical variables must be carefully controlled and the changes due to them must be taken into consideration when interpreting experimental results. Though these variables have been already discussed they may be restated.

1. The velocity of injection of the adrenalin solution must be carefully regulated. With extremely slow injections the maximum rise does not equal that produced by the same amount injected rapidly (2). A dilator effect may be converted into a pressor by the rapid injection of the same amount. Between the extremes, however, the degree of reaction is independent of the rate of injection.

2. Ventilation. Unless ventilation is kept constant the effect of adrenalin may vary. By decreasing aeration (3) a depressor effect may be converted into a pressor.

3. Blood pressure. Raising blood pressure by infusions decreases the response; lowering blood pressure by pithing increases the pressor response. A dose that causes relaxation and fall in blood pressure will, after pithing, cause vasoconstriction and a rise in blood pressure.

4. Anesthesia. Increasing the depth of the anesthesia increases the pressor response and may convert a depressor into a pressor effect (4).

5. Inertia (?). The first injection of adrenalin always produces a lesser effect than the second or third (chart 1). Similarly, the first

response after a long interval is less than the succeeding ones. Consequently to establish the norm at least three equivalent responses are necessary.

6. Blood volume. Rapid and great increases in blood volume cause a temporary increase in response (chart 2). Conversely, rapid losses of blood (hemorrhage), result in a temporary diminution.

With these variables controlled, Cushny (5) has estimated the accuracy of the test: "With due care the uniformity of the reaction of

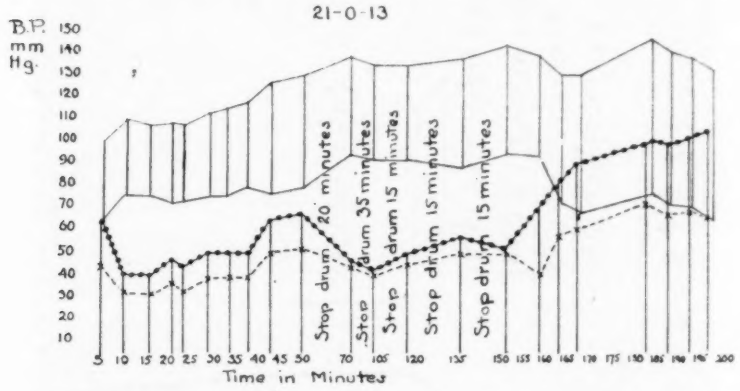

Chart 1. Single injections not sufficient to establish normal response to adrenalin. After period of rest the rise following first injection always less than that following second and third (inertia?). In the charts of this and subsequent articles, the time in minutes forms the base line and the blood pressure in mm. Hg. the abscissa. The upper solid line represents the systolic pressure at the height of the adrenalin response and the lower solid line the systolic pressure at the time of injection. The absolute and percentile increases in blood pressure calculated from these are represented by dash and crossed lines (--x--x--) and dotted lines (....).

the blood pressure to equal doses of adrenalin and its sensitiveness to comparatively small changes in dose are very remarkable." Elliott (6) says: "The circulatory system will respond with the accuracy of a chemical balance to any dose of adrenalin." Schultz (7) states: "It is remarkable how different sets of readings agree, how constant is the interval required for recovery, and with what certainty one can predict the height to which the blood pressure will rise."

From previously reported investigations we concluded that the response to adrenalin was a constant and that alteration in the response

following the introduction of any variable might be attributed to that variable. It soon became apparent, however, that in the majority of cases the response to repeated injections of similar doses was not a constant. Whether considered on an absolute or on a percentile basis, an increasing response appeared in from one to three hours after the first injection. This increase, once instituted, augmented progressively with each succeeding injection. In some experiments the increase

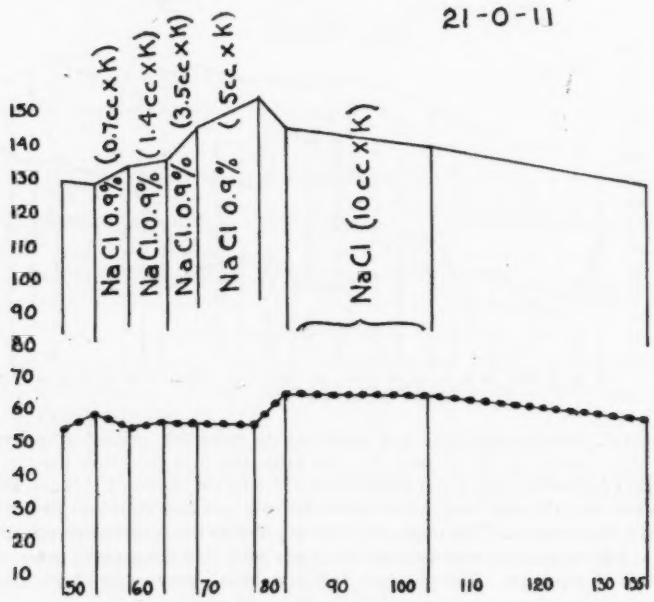

Chart 2. Effect of volume on adrenal in response. Even small quantities rapidly introduced cause slight, transient increase. Large quantities introduced slowly, without effect.

in response was more than 200 per cent (figs. 1 and 2; chart 3). This was quite independent of the general condition of the animal and was obtained with evidence of what we have called "tire" (i.e., long period of latency, plateau fastigium and gentle descending slope). In a few instances a depressor effect was converted into a pressor. In one experiment a diphasic effect was changed to a pure pressor (chart 4). The initial dilatation disappeared gradually and its loss was accompanied

by a progressive increase in the pressor effect. The phenomenon of progressively increasing pressor response to similar doses has been called, for want of a better term, "sensitization."

An investigation of the cause of this "sensitization" included the consideration of the following factors: *1*, Increase in blood volume; *2*, cumulation of adrenalin; *3*, stimulation of the adrenal medulla; *4*,

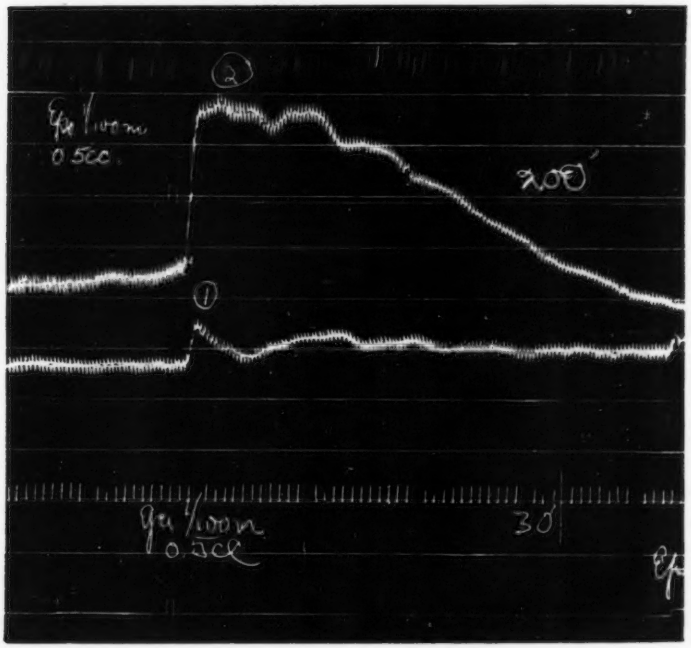

Fig. 1. Illustrating sensitization. Tracing 2 superimposed on tracing 1 after 170 minutes. Injections of adrenalin 1/100,000 (0.5 cc.) at signals. Greater response in no. 2 despite higher level of blood pressure.

stimulation of the thyroid; *5*, increased sensitivity due to parathyroid insufficiency.

1. Increased blood volume. The rapid introduction of large quantities of fluid (12 cc. per kilo) produced a slight transient increase in the reaction (chart 2), which disappeared within a few minutes and which differed entirely from the prolonged, gradually increasing effect seen in "sensitization."

2. Cumulation of adrenalin. A single injection of a large quantity of adrenalin (0.25 mgm.) (chart 5) did not increase the response to succeeding doses.

3. The rôle of the suprarenals. Ablation of the suprarenals during the progress of the sensitization put a stop to the increasing response (chart 6, B) but did not lower the existing plane of the reaction. It thus becomes apparent *a*, that while sensitization did not *continue* in the absence of the adrenals the height of the response could be main-

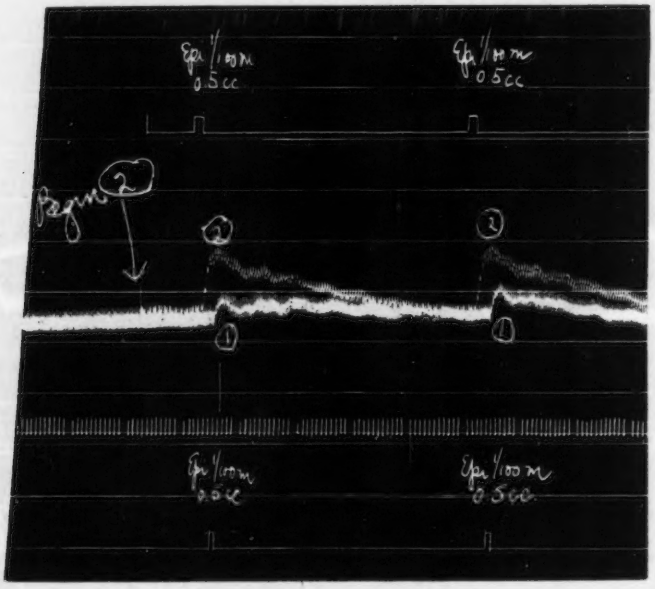

Fig. 2. Illustrating sensitization. Tracing 2 superimposed on tracing 1, made 80 minutes previously. At signals adrenalin 1/100,000 (0.5 cc.) injected.

tained; and *b*, that sensitization could be *initiated* in their absence (chart 6, A). Excision of such organs as the kidney or spleen after sensitization had been established produced the same effect as adrenalectomy. We concluded, therefore, that the inability to continue the sensitization after adrenalectomy was due to operative trauma and not to the absence of a specific hormone. Removal of the adrenals before any injections of adrenalin did not prevent the production of sensitization. It is therefore our opinion that the adrenals themselves play no more rôle

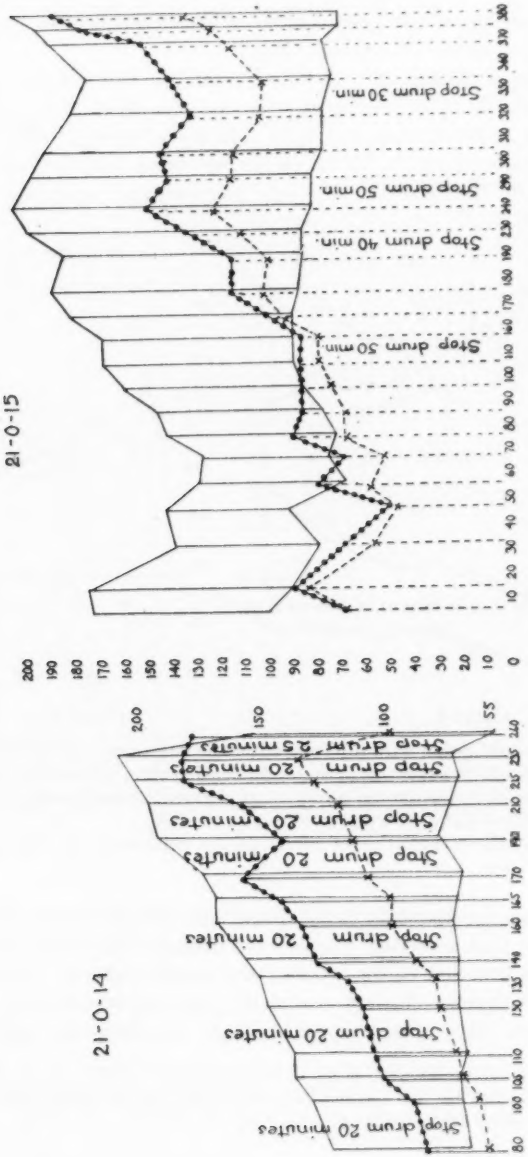

Chart 3. The progressively increasing response to repeated injections of equal doses of adrenalin (sensitization)

in the production of the phenomenon of sensitization than they do in the production of the von Anrep "step."

4. The rôle of the thyroid. In four animals the thyroid was removed[1] two and three days before the experiment. In all of these a clear-cut sensitization occurred (figs. 3, 4, 5, 6). In one of these cats the adrenals were also removed before the adrenalin injections, and the sensitization progressed in the absence of both thyroid and adrenal. Careful

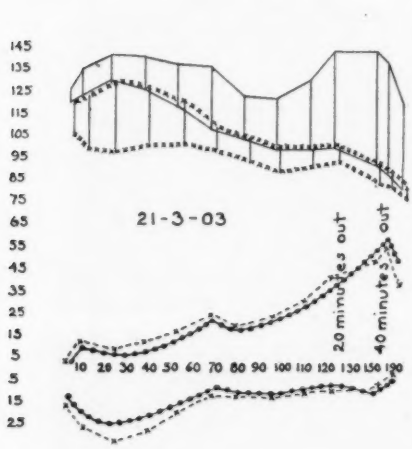

Chart 4

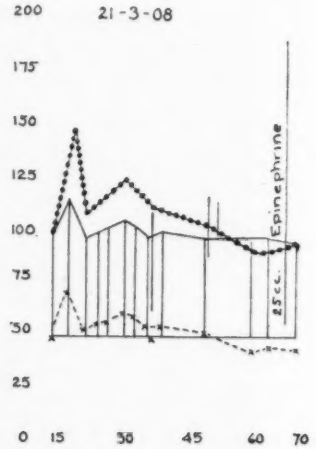

Chart 5

Chart 4. Sensitization with diphasic adrenalin response. Initial vasodilatation with secondary vasoconstriction. As sensitization proceeded the initial depression diminished while the constriction augmented until finally the dilatation was abolished and a pure pressor response resulted.

Chart 5. Neither cumulation of adrenalin nor volume change can alone produce sensitization.

autopsies from the mandible to the diaphragm revealed no thyroid tissue. It is possible that accessory glands were present; that these could have assumed the function of the whole gland seems unlikely. In order to rule out the thyroid element completely, one experiment was done on a cat fifteen days after thyroidectomy. A clear-cut sensitization

[1] The thyroidectomies were done in the laboratory of Dr. W. C. Clarke by Dr. Samuel Hirshfeld. We are also indebted to Doctor Hirshfeld for technical assistance in many of the other experiments.

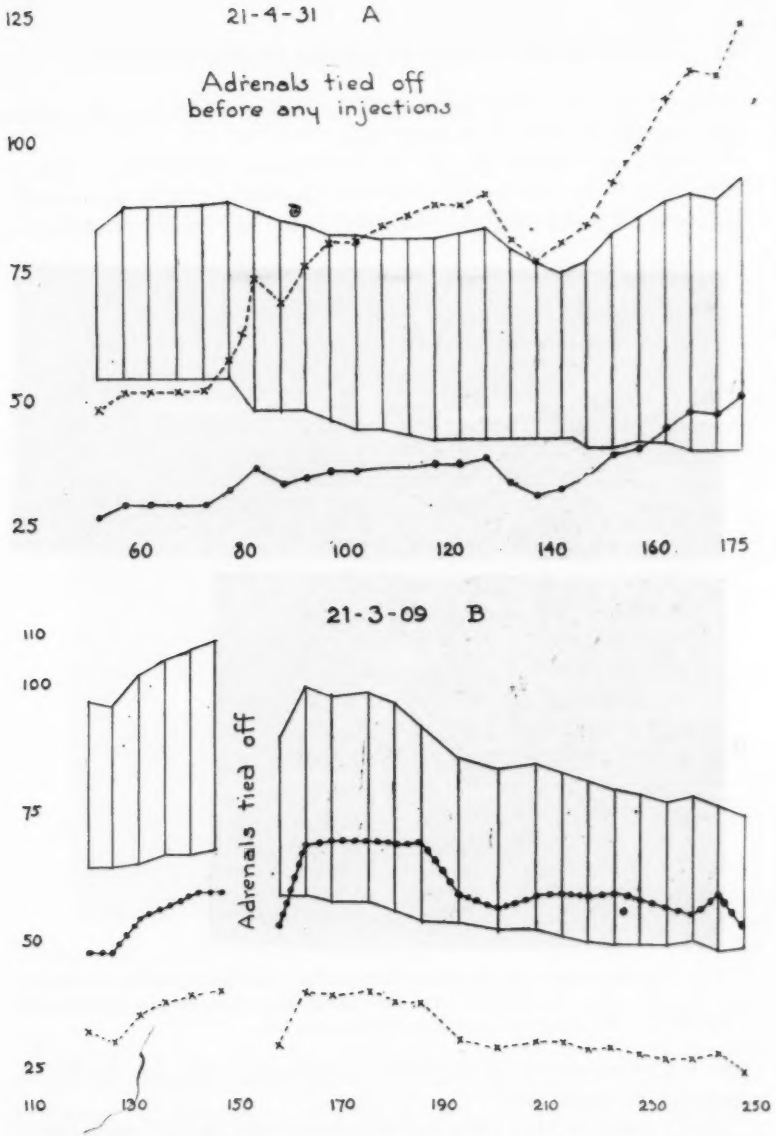

Chart 6. A: Sensitization occurring in animal whose adrenals were removed at the beginning of the experiment. B: Sensitization does not continue after removal of adrenals during experiment. That this is a traumatic rather than a specific effect is shown by the fact that sensitization is arrested similarly when kidney or spleen is removed. Note maintenance of blood pressure in absence of adrenals.

occurred (fig. 5). The cat showed the clinical picture of myxedema and autopsy revealed no thyroid tissue.

5. Increased sensitivity due to parathyroid insufficiency. Tetany developed in one cat from which the parathyroids were accidentally removed in performing thyroidectomy. In this animal sensitization

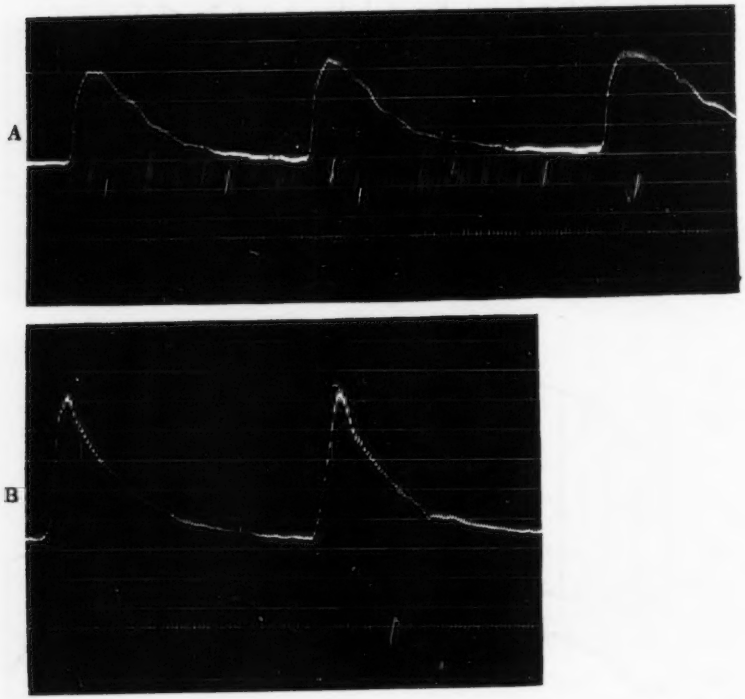

Fig. 3. Illustrating sensitization in animal that had been completely thyroidectomized 3 days previously. A, shows three control injections of adrenalin 1/100,000 and B shows two injections 170 minutes later.

was not obtained (chart 7); hence sensitization can not be due to parathyroid insufficiency.

Of the variables that could be held accountable for this sensitization, we have controlled the velocity of injections, the ventilation, the body temperature, and have excluded the factors of alterations of blood bulk and anesthesia, cerebrospinal changes, cumulative drug effect, stimula-

tion of the adrenal medulla or thyroid, and deficiency of the parathyroid secretion. The altered response must be due to a change at the site of action of adrenalin or at a point peripheral thereto. There remain, therefore, but two structures to be considered—the myoneural junctions of the thoracico-lumbar system and the smooth muscle fiber of

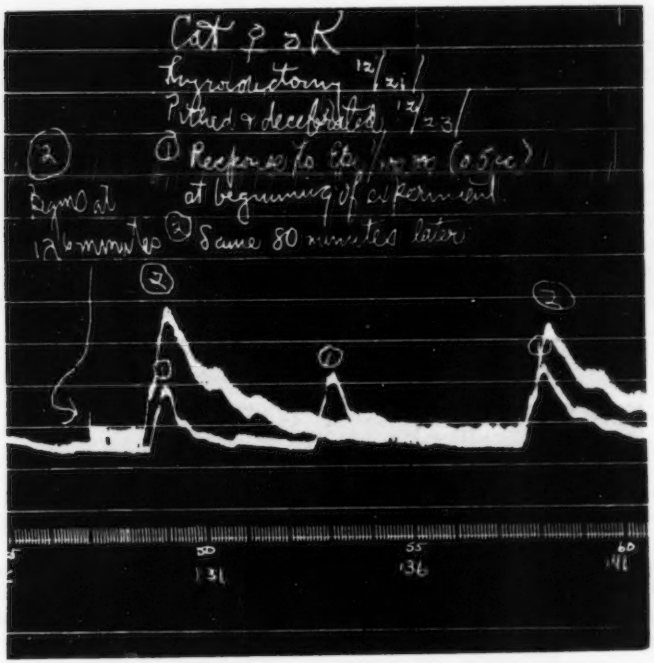

Fig. 4. Illustrating sensitization in animal that had been thyroidectomized 2 days previously. Tracing 1 shows three control injections of adrenalin. Tracing 2 shows two injections superimposed 80 minutes later.

the arterial wall. That the alteration is due to change in the muscle fiber (lengthening) seems unlikely in the presence of a constant blood pressure. It seems most likely that the sensitization is due to an alteration in the myoneural junctions. We are, at present, ignorant of the nature and causation of this change. In a paper to follow a report will be made of several factors that have been investigated.

The bearing of sensitization in the interpretation of previous work done with the same preparations. We have shown that, in the response of the involuntary nervous system to repeated injections of adrenalin, there is present in many, but not all, animals a constant variable. Other investigators have regarded the response of the involuntary nervous system as a constant. It was necessary for us, therefore, to determine whether sensitization occurred in their experiments and to analyze their interpretations. In the published work of Cushny (5), Schultz (7), Barger and Dale (8), Hunt (9) and Elliott (6), the injections were

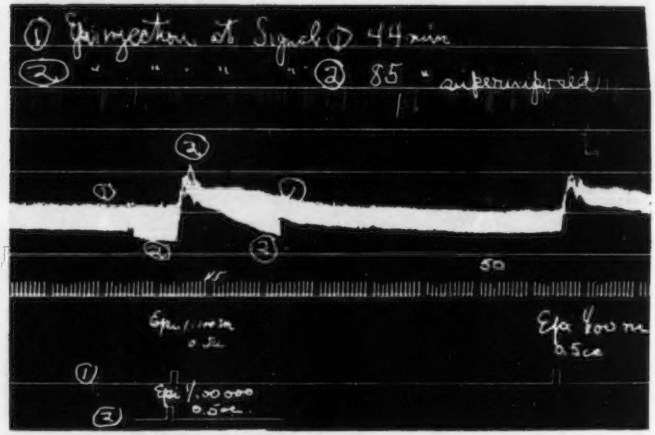

Fig. 5. Illustrating sensitization in animal that had been thyroidectomized 15 days previously. Tracing 2 superimposed on tracing 1 after 41 minutes. Though blood pressure level of no. 2 is slightly lower than no. 1 the height of the response is greater both absolutely and on percentile basis.

not repeated often enough to permit sensitization to occur. Hoskins (10) and his colleagues compared the responses to adrenalin in anesthetized dogs before and after the introduction of a variable, such as the removal of a ductless gland. The involuntary nervous system was regarded as a constant, and alterations in the adrenalin response were attributed to the variable. While this work was carefully controlled, it seems extremely hazardous to interpret alterations in the adrenalin response in the presence of so many variables: alterations in the depth of anesthesia, in the vascular tonus, in aeration, and especially in the irritability and tonus of the involuntary nervous system itself. Ostwald

(11) and Ascher and Flack (12) have reported similar experiments. Ostwald, however, publishes no experimental results, and those of Ascher and Flack are unconvincing because of the irregularity of blood

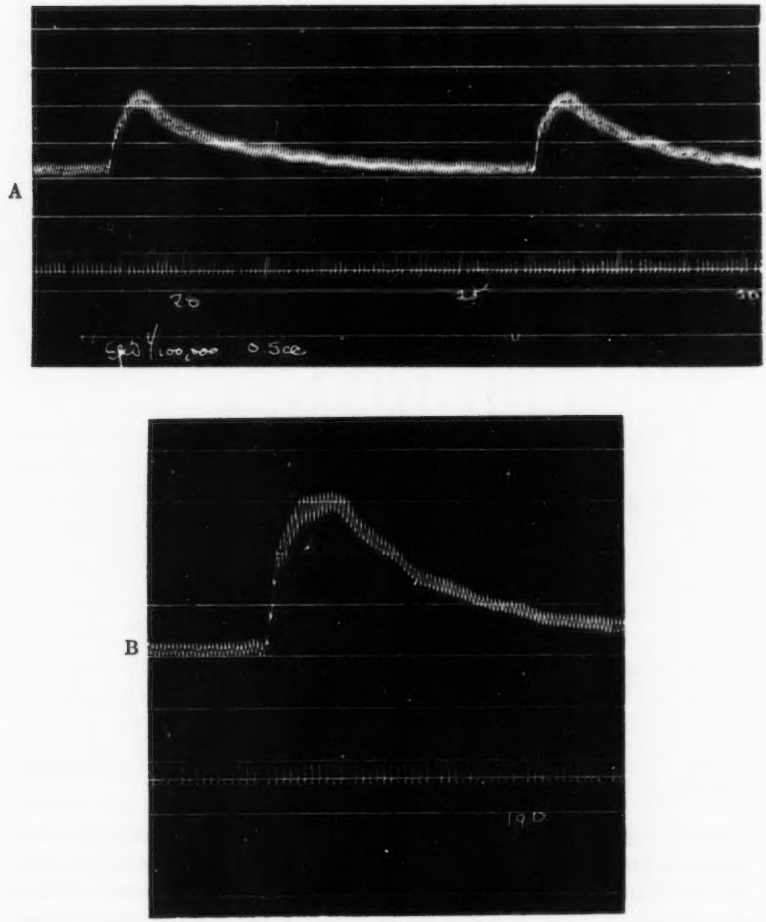

Fig. 6. Illustrating sensitization after thyroidectomy 4 days previously. First tracing A, shows two injections at 20 and 25 minutes. Second tracing B, one injection at 190 minutes.

pressure and adrenalin response; their conclusions are based on very slight differences in response.

Using a technique similar to ours, except that he pithed only to the level of the fourth thoracic vertebra, Levy (13) has published findings which we are able in the main to confirm, but in the interpretation of which we differ from him. He has described sensitization occurring after a latent period from stimulation of the cervical sympathetic,

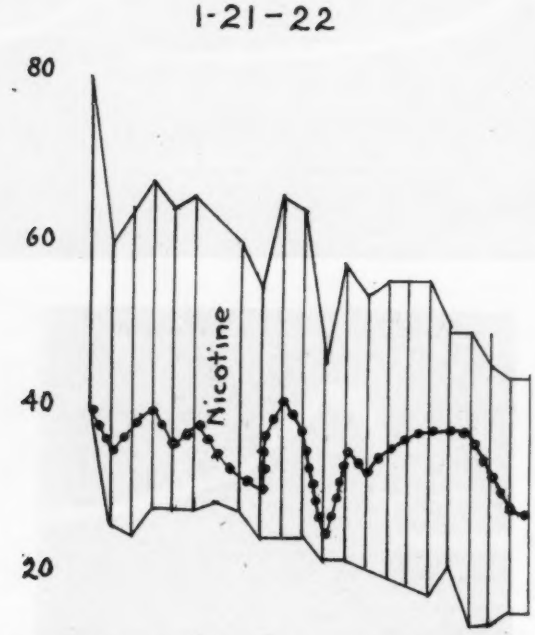

Chart 7. Following thyroidectomy, cat develops tetany. No sensitization to adrenalin in the presence of active tetany.

from the injection of thyroxin and of large doses of adrenalin and from asphyxia and excitement. He regards the involuntary nervous system as a constant and postulates a variation of the thyroid hormone. When the cervical sympathetic was stimulated and the thyroid gland immediately removed, sensitization occurred after a latent period. The delayed sensitization is interpreted as due to a gradual breaking down of the thyroid secretion resulting from the stimulation of the cervical

sympathetic. Our experiments differ from those of Levy in that he claims to have obtained specific sensitization with thyroxin. Our experiments lead us to conclude that his interpretation of sensitization is not justified, since he regarded the involuntary nervous system as a constant. That the sensitization is not due to thyroid secretion is evident from our experiments with thyroxin. Not only do our experimental data point strongly to the fact that the thyroid hormone plays no part in the sensitization, but our general knowledge of the physiological action of thyroxin is contrary to the belief that a thyroxin effect can be manifested within a few hours, as Levy would have us believe. As evidenced by the elevation of basal metabolism, the effects of thyroxin do not usually occur for 72 hours. Marine (14) worked upon the effect of adrenalin upon basal metabolism in animals with and without thyroid glands, and he reports an inability to discover a thyroid effect in acute experiments. This disparity in results we believe is due to the erroneous interpretation of Levy and of Ascher and Flack, and we agree fully with Marine that in acute experiments there is no demonstrable evidence of a synergism between thyroid extract and adrenalin.

The practical application of these data is to be found in the discussion of the pathogenesis of Graves' syndrome. The work of Levy, of Ascher and Flack and of others, has been taken to show *1*, that the thyroid hormone circulates in the blood; *2*, that the cervical sympathetic is the secretory nerve for the thyroid gland; *3* that stimulation of this nerve causes an immediate secretion; and *4*, that a synergism exists between the secretions of the adrenal medulla and of the thyroid gland. If these deductions are accepted, the explanation of the pathogenesis of Graves' syndrome is very obvious. One postulates a production of a sympathomimetic syndrome by a synergism of thyroid and adrenal medulla hormones. As corollaries follow: sensitiveness to adrenalin is due to hyperthyroidism (as popularized by Goetsch) and thyroid enucleation is curative. But our data tend to show that, in acute experiments at least, the sensitiveness to adrenalin is not due to changes in the thyroid, but to changes in the involuntary nervous system. We have failed to find any evidence of synergism between adrenal medulla and thyroid gland. It should be clearly understood that we do not insist that the deductions advanced by other workers are necessarily erroneous, but we do insist that the data upon which these deductions are based can be differently interpreted. These facts and theories will be referred to again in the papers on natural and artificial sensitiveness to the subcutaneous injections of adrenalin.

CONCLUSIONS

1. In the vascular response to the repeated injections of similar doses of adrenalin, all known variables being carefully controlled, there is present in most, but not in all, animals a constant variable.

2. This variable consists of a progressively increasing response.

3. This variable is not due to cumulation of adrenalin, increase of blood bulk, stimulation of thyroid or adrenal medulla or parathyroid insufficiency.

4. This variable is frequently present when the animal shows evidences of "tire" and is definitely not associated with hypertonicity of the involuntary nervous system.

5. This variable has not received consideration in the literature. In work similar to our own, the involuntary nervous system has been considered a constant, and this sensitization has been interpreted as the result of some artificially introduced variable.

6. The bearing of these data on the clinical problem of Graves' syndrome and of the Goetsch test is briefly indicated.

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STUDIES OF GRAVES' SYNDROME AND THE INVOLUNTARY NERVOUS SYSTEM

VI. Attempts to Alter the Vascular Response of the Pithed Cat to Repeated Injections of Similar Doses of Adrenalin

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In a previous paper (1) we discussed the "sensitization" of the involuntary nervous system following repeated injections of adrenalin; the effects of several classes of drugs and chemicals were next investigated to determine their effect on the thoracico-lumbar system. In order that a drug may decrease the adrenalin response it must first overcome the effects of sensitization. Sensitization rendered extremely difficult the interpretation of the effects produced by substances reported to increase the response to adrenalin. It was necessary to determine whether we were dealing with a non-specific sensitization, with a specific effect, or with a combination of the two.

1. Buffer salts. Sodium acid phosphate (10 per cent) in doses of from 2 to 11.6 cc. per kilo of cat caused a slightly increased response to adrenalin. This may have been a volume effect.

Sodium carbonate (20 per cent), 5 cc. per kilo, caused a decreased response, which was very slight and transient.

Sodium bicarbonate (9.6 per cent), 6.3 cc. per kilo, caused a slight evanescent decrease in response—too small to be significant. It seems, therefore, that attempts to alter buffer interplay do not result in a definite modification of the response to adrenalin. Collip (2) has reported an increased pressor response to adrenalin after sodium bicarbonate and a decrease after sodium acid phosphate. Our results accordingly are diametrically opposed to those of Collip. His published plates show such an irregular blood pressure that his experiments may be justly criticised. His normal is based on an insufficient number of

control adrenalin responses, and he fails to take into consideration the volume effect. In all probability the rates of injection varied widely, for it is impossible to expel large amounts of fluid from a syringe at a constant rate. Collip worked with anesthetized animals whose cerebrospinal axes were intact, thus introducing two variables which were absent in the preparations employed by us.

2. Alkaloids. Atropine and physostigmine in doses sufficient to produce pharmacological effects failed to alter the response to adrenalin. Morphine was without effect. Cocaine (1.0 mgm.) caused a striking increase in the response to adrenalin.

3. Anions and cations. From the results of Crile's (3) work with the iodides it was expected that iodine would greatly increase the vascular response to adrenalin. Our experiments failed to confirm this expectation. In numerous experiments large doses of sodium iodide were repeatedly injected and in no instance could we satisfy ourselves that the iodine modified the response to adrenalin. When increased reactions occurred it was either a volume effect or a sensitization (chart 1). These results are opposed to Crile's exposition of the pathogenesis of Graves' syndrome. Crile has advanced the theory that the primary etiologic factor in Graves' syndrome stimulates the thyroid; the output of its iodine-containing hormone is increased and the iodine so secreted stimulates the involuntary nervous system. This stimulation accounts for the familiar sympathomimetic symptoms. He believes that the thoracico-lumbar transmits secretory impulses to the thyroid and he describes a vicious cycle which he interrupts by thyroid extirpation.

Bromides were next investigated because of their widespread use in Graves' syndrome. More than one gram per kilo did not alter the response to adrenalin.

Magnesium sulphate produced a striking and marked decrease in the response to adrenalin (chart 2). In doses of from 100 to 150 mgm. per kilo by vein it caused pronounced effect. This dose depresses neither heart nor respiratory center and did not alter blood pressure.

The effect of the sulphate ion was controlled by the injection of sodium sulphate.

The injection of large doses of calcium chloride was without effect on the adrenalin response. The opposite type of experiment in which the blood calcium was precipitated with sodium citrate indicated that decreased blood calcium did not modify the usual reactions to adrenalin.

Potassium chloride, in doses sufficient to depress the myocardium, had no effect on the response. It may therefore be concluded that Na,

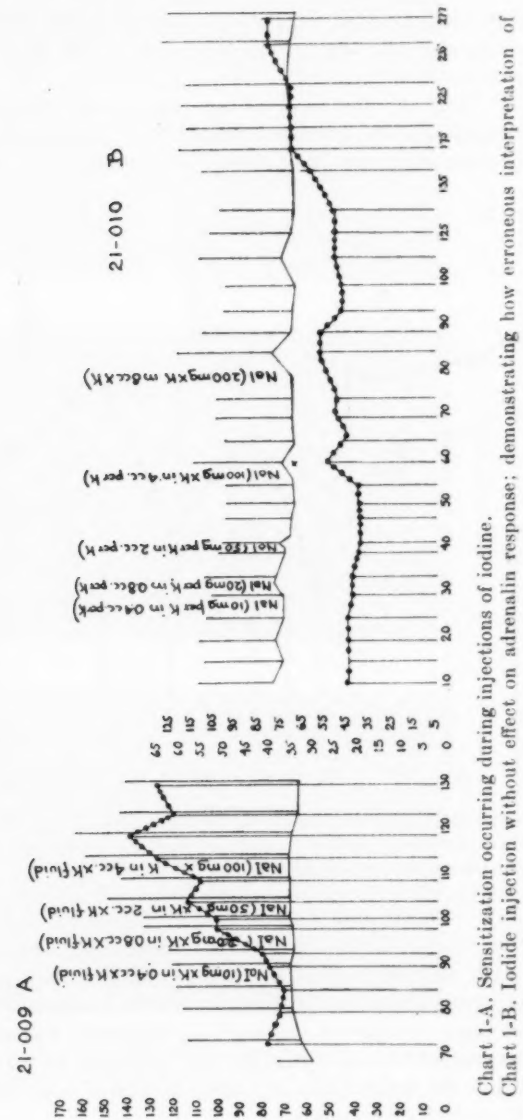

Chart 1-A. Sensitization occurring during injections of iodine.

Chart 1-B. Iodide injection without effect on adrenalin response; demonstrating how erroneous interpretation of results may be made.

K, Ca, I and Br, SO_4, Citrate, Cl, H_2PO_4, CO_3, and HCO_3 are without effect on the response, and that of the ions studied Mg alone gives a demonstrable alteration.

4. Hormones. Levy (4) has reported an "immediate sensitization" following the exhibition of thyroxin. Marine's (5) laboratory studies on basal metabolism show that the earliest effect from thyroxin does not

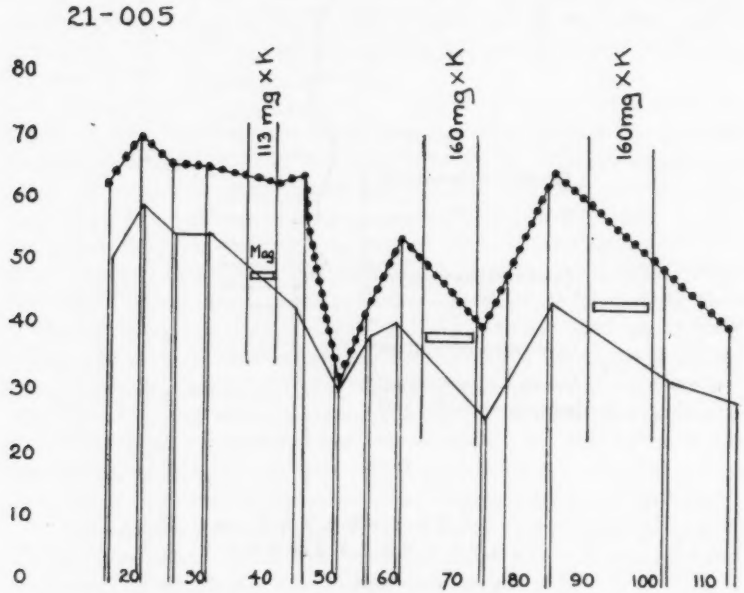

Chart 2. Magnesium sulphate causes striking decrease in pressor response to adrenalin. Effect is transient and is accomplished by doses insufficient to depress arterial muscle or the respiratory center.

NOTE:—See chart 1, article V, for explanation of chart.

appear until at least eight hours after administration. From many experiments with pure crystalline thyroxin obtained from Squibb & Company and with "Thyroidin" (Merck) we were unable to convince ourselves that these substances augment the adrenalin response (fig. 1). In one experiment no sensitization was obtained at all, and in experiments where the sensitization did occur it differed in no wise from that found in control experiments.

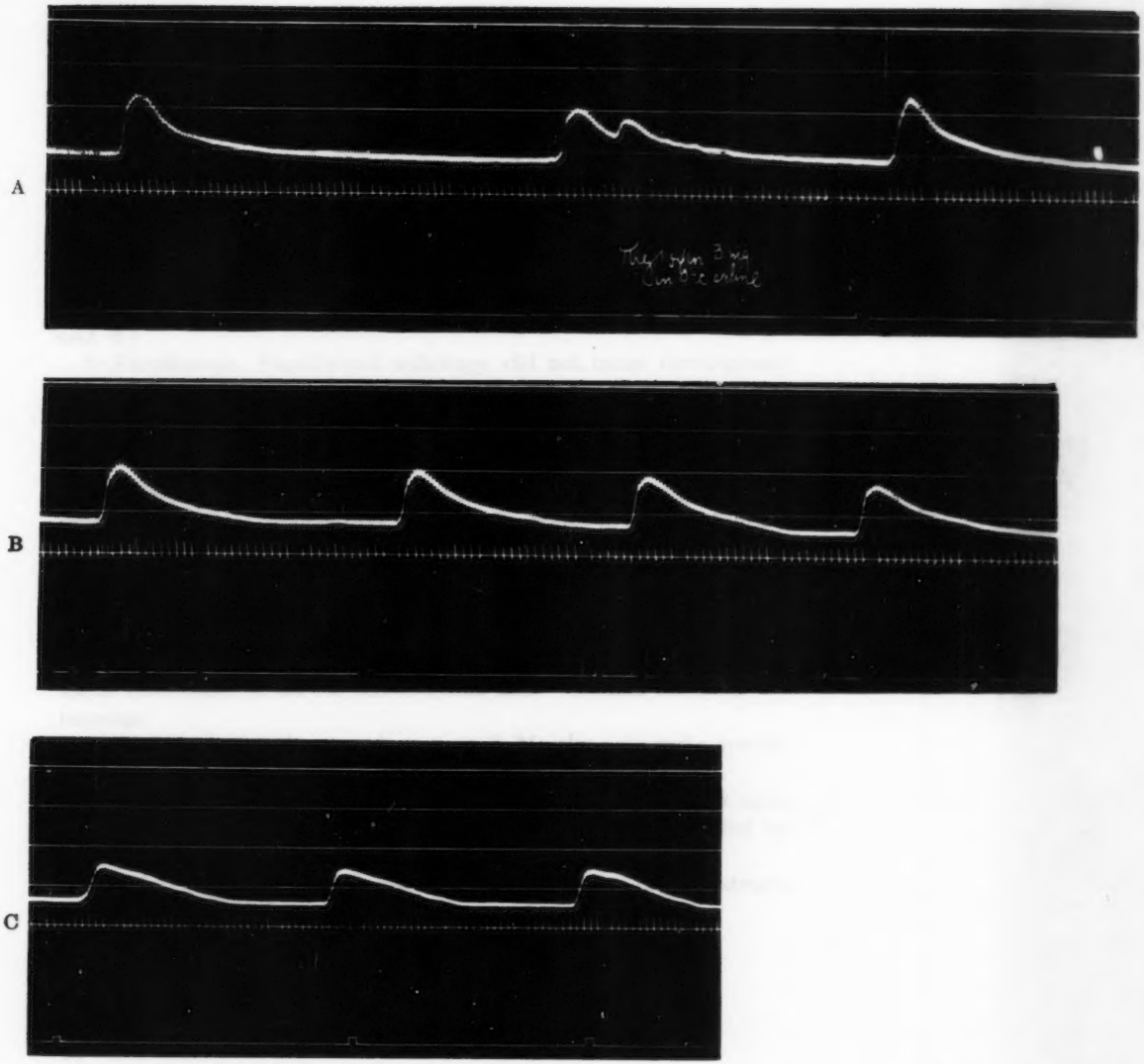

Fig. 1. One of many experiments showing that crystalline thyroxin introduced intravenously has no effect on the adrenalin response, in acute experiments at least.

Pituitary extract was without effect. Adrenalin infusions have already been discussed. (Article V, chart 5.)

5. Extirpation of the suprarenal glands. In a previous paper extirpation of the suprarenal glands has been shown to be without apparent effect on the sensitization. (Article V, chart 6.)

6. Extirpation of the thyroid. Experiments previously reported indicate that, contrary to the findings of Levy, sensitization may develop and persist in the absence of the thyroid gland. The presence of myxedema does not prevent sensitization. (Article V, figs. 3, 4, 5 and 6.)

7. Parathyroids. Parathyroid deficiency did not cause development of sensitization. (Article V, chart 7.)

CONCLUSIONS

1. Before factors that alter the adrenalin response can be given their proper value, the "sensitization" due to alterations in the involuntary nervous system must be eliminated.

2. Before a substance can decrease the adrenalin response it must overcome the effects due to sensitization.

3. Introduction of buffer salts does not alter the adrenalin response.

4. No alteration followed drugs acting selectively on the craniosacral mechanism or on the cerebrospinal axis. Cocaine increased the response.

5. Of the anions and cations investigated, Mg alone showed a specific effect: a diminution of the response.

6. Iodides failed to alter the response. The bearing of this fact upon the theory of the pathogenesis of Graves' syndrome as enunciated by Crile is discussed.

7. Ablation of the endocrine glands or the injection of potent extracts of these glands failed to modify the response to adrenalin.

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STUDIES OF GRAVES' SYNDROME AND THE INVOLUNTARY NERVOUS SYSTEM

VII. On the Mechanism of Sensitization to Subcutaneous Injections of Adrenalin

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Sensitization to parenteral injections of adrenalin has been extensively studied in the past few years. Following the work of Ascher and Flack (1) and of Levy (2) who demonstrated an increasing pressor response to adrenalin, which they considered due to synergistic action of the thyroid secretion, Goetsch (3) suggested that this phenomenon might be of value in the diagnosis of thyroid disease. In his later writings, Goetsch altered his position to the statement that while the adrenalin is indicative only of alterations in the sympathetic system, it was nevertheless of great value particularly in the diagnosis of thyroid disease. Today many clinical workers place great reliance upon this test, and in certain clinics it is regarded as pathognomonic of "hyperthyroidism."

Our work on the mechanism of the sensitization produced in pithed cats by the repeated intravenous injection of equal doses of epinephrin suggested that it might be possible to produce sensitization to adrenalin injected subcutaneously, and that the data referable to the intravenous sensitization would hold equally well for the subcutaneous sensitization.

Experiment. Using the pithed decerebrate cat preparation previously described, we were able to produce a clear-cut response to adrenalin injected subcutaneously in animals who previously had failed to respond (figs. 1 and 2). The sensitization, however, could not be produced with

[1] Though the name of C. S. Spencer, Research Assistant in the Department of Pharmacology, does not appear as co-author in any of these articles, she has, by her technical skill, extraordinary knowledge of her subject and her clear thinking, given unusual help in the development of the protocols, the experimentation and the publication.

any degree of regularity. In many of the animals no effect whatsoever could be obtained. In other animals, while no immediate response to the injection was obtained, there occurred large waves on the blood pressure tracing (figs. 3 and 4), which were not present when no adrenalin was injected, even after many hours, and which differed from the small intrinsic waves which we have previously described, in that they were

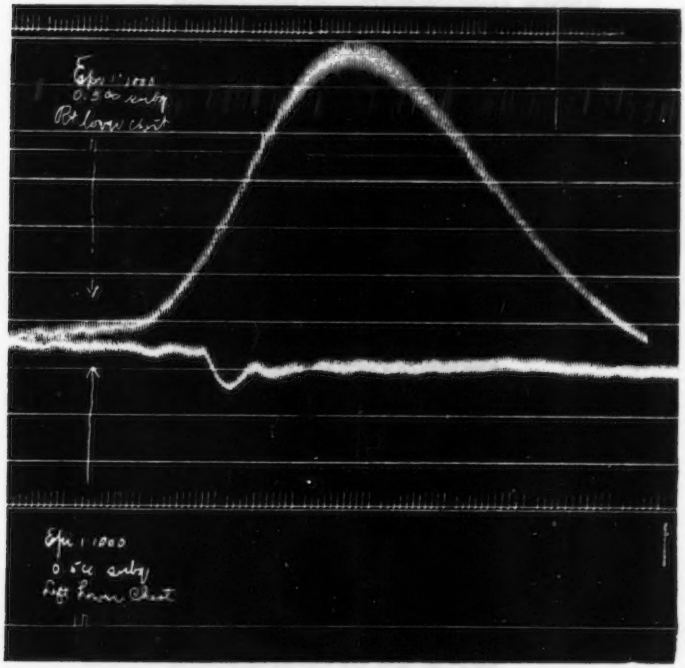

Fig. 1. Illustrating sensitization to subcutaneous injection of adrenalin 1/1000 (0.5 cc.). Figure 2 (injection into right chest) superimposed on figure 1 (injection into left chest) after 5 hours.

more extensive and of greater duration. In some of the animals,where a marked and immediate response was obtained, repetition of the injection within a few minutes was usually followed by a negative result, so that at first we thought that the initial response might be due to some technical error. The injections were therefore made with the most unusual care, and gentle palpation made over the site of injection to

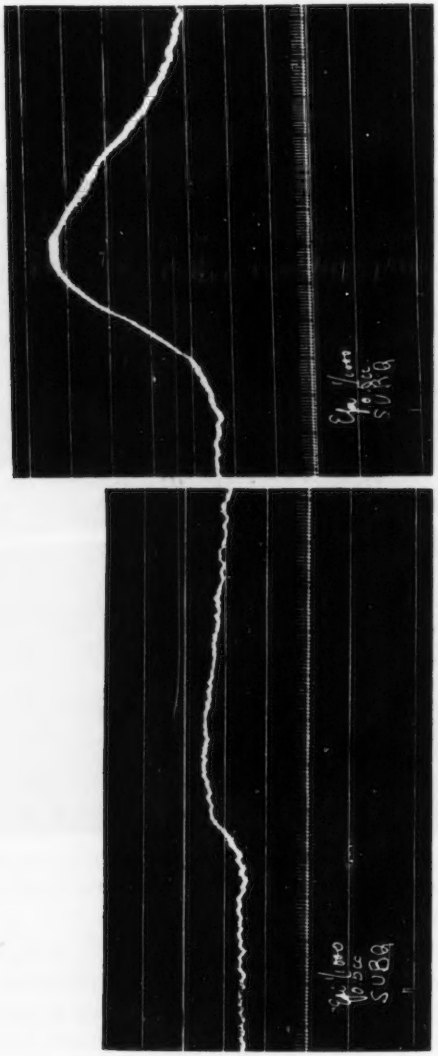

Fig. 2. Illustrating sensitization to subcutaneous injection of adrenalin. First tracing shows slight reaction and second a marked reaction one hour later. Nothing done in intervals between injections.

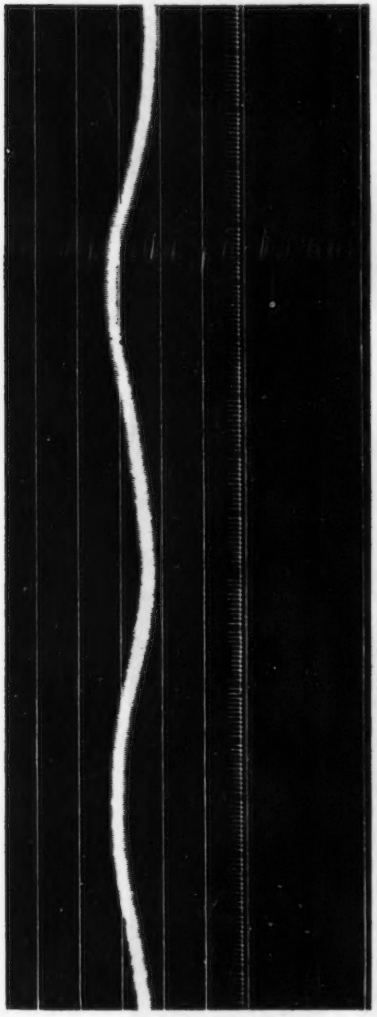

Fig. 3. Illustrating waves occurring after the subcutaneous injection of adrenalin. 22–2–4. Cat F K. Decerebrated and pithed. No injections for one hour, during which time blood pressure tracing was absolutely level. One injection subcutaneously of adrenalin 1/1000 (0.5 cc.) causes no alteration in blood pressure. One hour after this these waves appear. In tracings that have run for many hours if adrenalin is not at any time used, these waves do not appear.

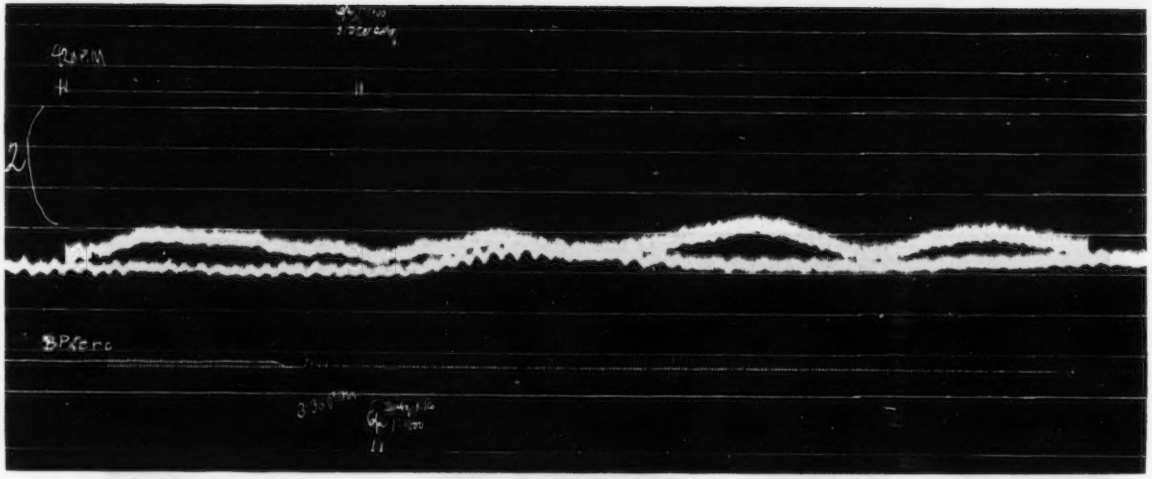

Fig. 4. Illustrating waves following subcutaneous injection of adrenalin. Tracing 1 shows curve as it had appeared for the five preceding hours since the operation. Only the intrinsic waves are present. Following the subcutaneous injection of adrenalin at 3:30, the beginning of the larger waves can be seen. Tracing 2, superimposed one hour later, shows these waves well developed though no reaction to adrenalin occurs at 4:30 when the drug is introduced subcutaneously again.

ascertain whether the adrenalin had infiltrated the tissues. The experimenters felt perfectly satisfied after all these precautions that there was no technical error responsible for this inexplicable phenomenon.

CONCLUSIONS

1. In cats prepared as described, in whom the first subcutaneous injection of adrenalin causes no reaction, a clear-cut reaction may be obtained at times upon succeeding injections.

2. This sensitization to the subcutaneous injection of adrenalin is inconstant.

3. It is fair to assume that the mechanism of production of the subcutaneous sensitization is not unlike that of the intravenous sensitization.

4. Inasmuch as the sensitization to the intravenous injections is absolutely independent of the thyroid gland, it is fair to assume that sensitization to the subcutaneous injection of adrenalin is also independent of the thyroid gland.

5. There is no scientific basis for the value of the so-called Goetsch test in the diagnosis of thyroid disease.

6. The sensitization to adrenalin is probably due to an alteration in the peripheral structures of the involuntary nervous system.

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MEASUREMENTS OF PULSE WAVE VELOCITY

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It has long been recognized that it would be extremely valuable if even a rough estimate of the circulation rate could be made from blood pressure measurements and in attempting to do this various figures have been used, the formula most commonly employed being the product of the pulse rate and pulse pressure originally suggested by Dawson, Higgins and Gorham (3), (4). It is however clear that the elasticity of the arteries must play a most important part, and that allowance for this must be made if any such calculations are to be reliable. In consequence modifications of the above formula have been suggested, and the output of the heart per beat has been taken as being proportional not simply to the pulse pressure but to the quotient $\frac{\text{pulse pressure}}{\text{systolic pressure}}$ as suggested by Strasburger (14), or to the quotient $\frac{\text{pulse pressure}}{\text{diastolic pressure}}$ which was put forward by Stone (13).

One of us (H. C. B.) (1) in a preliminary communication published during the war suggested that the blood pressures obtained clinically seemed more reasonable if the quotient $\frac{\text{pulse pressure}}{(\text{systolic pressure})^2}$ was used rather than the simpler $\frac{\text{pulse pressure}}{\text{systolic pressure}}$ figure. The whole question therefore demands further investigation, since all these formulae are based on slender evidence, and the direct measurement of elasticity during life by the determination of pulse wave velocity has been very largely neglected.

As long ago as 1878 Moens (12) showed that the elasticity of the blood vessels as measured by pulse wave velocity varied with the blood pressure, and Grunmach (8) shortly after this confirmed his observations and also showed that the pulse wave velocity might show considerable variations in man. Dawson (5), however, in 1917 investi-

gated in animals the relationship between blood pressure and pulse wave velocity and found no consistent relationship between them. This in reality is not surprising since the blood pressure will be influenced by the aggregate effect of the varying elasticities of different vessels, while the pulse wave velocity is judged by measurements along a single vessel. With vessels having such a pronounced muscular coat as arteries it is not to be expected that their elasticity will depend *only* on their internal pressure, though this is likely to be one factor, and consequently discrepancies must be exp. cted. On the other hand it mush be remembered that any contraction of the muscular coats, if occurring in many vessels, is bound to have some effect on the blood pressure.

Consequently some figures for pulse wave velocity in normal subjects, together with a few observations on hospital patients have been collected by us during the last three years, most of these observations having been made in the course of demonstrations of electrocardiographic methods to students, and they are here brought forward because the subject is now receiving some attention (A. V. Hill (10) and Bramwell and A. V. Hill (2)) and we consider that our data demonstrate not only the need for very careful choice of accurate methods but also of simultaneous records from several vessels, since the pulse wave velocity is not constant, but is very considerably slower in the larger central vessels than in the smaller peripheral branches.

TECHNIC EMPLOYED. The polygraph was discarded, proving incapable of sufficient accuracy, and a trial was then given to the comparison of radial and carotid records obtained by using recording tambours covered with very light but tensely stretched rubber and provided with very short and extremely light levers of straw pared down with a razor, the levers being placed so that they lay in the beam of light of an Einthoven string galvanometer. In this way shadowgraphs were obtained on the moving plate of the camera which could be read to the nearest 0.01 second. A greater accuracy than this was not obtained owing to the remaining instrumental inertia rounding off the commencement of the curves. The length of the vessels was estimated by measuring from the angle of Ludwig to the position of carotid tambour and also from the same angle along the course of the brachial and radial vessels. For the latter measurement the most accurate method was found to be the abduction of the arm through an angle of 90° and the measurement of the distance from the angle of Ludwig to the tip of the acromion process and from there to the antecubital fossa for the brachial or to the wrist for the radial.

Measurements made in this way on a body in the dissecting room had an error of ±1 cm. or less.

Using carotid records obtained in this way it was at once found that the sphygmic oscillations which were shown by Frank (7) to occur in the central vessels during the period of rising tension were often visible in the carotid records and yet might not be sufficiently well differentiated by this method to make reading of the main carotid wave easy. Attempts were then made to take simultaneous records of the apex beat, brachial and radial, to allow deductions of the pulse wave velocity, and it was in this way noticed that the transmission rate was very much slower in the larger central vessels than in the peripheral. It is on this fact that we wish to lay particular emphasis and hence the methods here used must be further detailed.

The apex beat record was obtained by the method used by Weitz (15) with slight modifications, but a recording tambour with a very light lever giving a shadowgraph was used instead of a Frank capsule and mirror. A tambour of 1.5 to 2.0 cm. diameter was employed, which was covered tightly with very light rubber, and connected with a light axle (weight 0.5 gm.) and with a light straw lever of 7 cm. length and weight 0.01 gram. Such a tambour had a vibration frequency of 20 to 30 per second, but its own vibrations were rarely visible on the records and simultaneous records of electrocardiograph and apex beat, brachial and radial pulses could be obtained quite simply. For the receiving tambour for the apex beat we have used a tambour of about 4 cm. diameter covered with rubber and held over the apex by the use of a felt pad and two elastic bands, one passing over the right shoulder and the other round the chest wall, in this again closely following Weitz.

It is obvious that the above technic is not ideal, since there is admittedly still an element of inertia in the recording system, but by careful attention to details we have not found these errors serious and our records have shown a very close resemblance to those obtained by Weitz with the Frank capsule. In addition more recently one of us (H. C. B.) has collected a few more figures using Frank capsules (Wiggers pattern) with light mirrors for apex beat, brachial and radial and making careful allowance for parallax, and the results previously obtained have been amply confirmed. Dr. Jane Sands, using Frank capsules, has also very kindly made a number of measurements for us of the pulse wave transmission time in the arm on some of the University of Pennsylvania medical students during class demonstrations, and some of her figures are included in this paper. Two of her records are also reproduced.

Using the Frank capsules and making enlargements of the photographs, readings may easily be made with an error of ± 0.003 or less in many cases. The transmission time from the receiving to the recording tambours was with the lever records considerably less than 0.01 and with the Frank capsules and longer connecting tubes was 0.010 second, and allowance has been made for this.

While the method has been technically fairly accurate the interpretation of apex beat records remains a controversial point. In our opinion it is often impossible to read an apex beat record unless it is controlled by a simultaneous electrocardiographic curve, with which the apex beat changes may be correlated. We have found that slight changes in the position of the subject may greatly influence the character of the apex beat curves, this being no doubt due to the complicated character of these curves, formed as they are by combinations of volume changes of the heart with the effects of changes in the heart's position. We have therefore followed Weitz in the interpretation of the curves produced, but have controlled our interpretations by the assumption that the curve on the apex beat record corresponding with the commencement of the expulsion period of the heart must occur under normal conditions within from 0.05 second to 0.07 second after the peak of the R wave. Unless the apex beat did show a definite wave corresponding to about this point the expulsion period has been taken as commencing 0.06 second after the summit of the R wave, since Weitz gives 0.06 second as the average rising tension time in normal adults. We do not imagine that anyone will deny that we are unlikely to be more than 0.01 second or 0.02 second at the most in error in estimating the commencement of the expulsion period in this way. It will be seen later that though such an error would of course alter our figures yet it would not be of sufficient magnitude to influence at all our deductions as to the great variability in pulse wave velocity in vessels of different size and position.

Lastly, all our observations have been accompanied by measurements of the blood pressure in the arm by the Riva Rocci method, using the auscultatory criteria. The blood pressure measurements were usually made several times both before the record was taken and directly after, though in a few cases two or three readings immediately after the obtaining of the record were alone made. The diastolic pressure has been taken as that pressure at which the sounds change from the third to the fourth phase and begin to appear more distant.

RESULTS OBTAINED: *Comparison of carotid and radial pulses.* So many figures are available in the literature for pulse wave velocity

measuring from these points that little emphasis need be laid on these results. In table 1 are collected some of our figures, in which the records were fairly clear, selection having been made to give records on individuals over a wide range of systolic and diastolic pressures. It will be seen that in those individuals in which the vessels may be presumed to be normal the velocities obtained vary between 5.2 and 7.9 meters

TABLE 1

Carotid and radial

	SEX	AGE	BLOOD PRESSURE	PULSE RATE	WAVE VELOCITY	REMARKS
1	M.	9	92/72	118	6.0	
2	F.	13	96/74	127	5.2	Tuberculous glands
3	M.	14	116/75	63	6.0	
4	M.	15	106/80	74	7.6	Mitral regurgitation
5	F.	20	116/76	75	6.3	Athletic
6	F.	22	120/70	95	6.4	
7	F.	23	116/70	79	7.4	
8	F.	23	108/65	65	5.7	Athletic
9	M.	23	168/35	70	6.3	Aortic regurgitation
10	F.	26	104/60	96	5.5	
11	M.	26	106/78	49	6.9	
			98/73	67	5.7	After nitroglycerine
			90/70	90	5.8	After nitroglycerine
			105/75	83	6.6	After tyramine
12	F.	30	94/66	60	6.1	
13	M.	34	119/92	107	8.8	Syphilitic arteritis
14	M.	36	122/84	73	7.9	Neurotic
15	M.	40	128/76	82	9.0	Shortness of breath
16	M.	45	118/62	86	6.5	Splenic anemia, hemoglobin 35 per cent
17	M.	57	205/125	62	12.4	Arteriosclerosis
			183/123	68	11.0	After nitroglycerine
18	M.	65	84/60	84	8.1	First day up after operation for strangulated hernia
19	F.	67	142/86	100	13.3	Arteriosclerosis retinal changes
20	M.	75	150/80	70	9.8	Arteriosclerosis

per second, while the average for the whole table (omitting cases 13, 15, 17, 18, 19 and 20 in which arterial disease is probable) is 6.35 meters a second. This is a somewhat lower figure than is usually given as normal, but this table contains a considerable proportion of children and young women, many with rather low blood pressures. In table 2 are given a number of observations on the same subject with experimentally

produced changes in blood pressure. Here the average figure for normal conditions is 7.3 meters a second and of all determinations 7.7 meters a second. (Nitroglycerine was given in 1 per cent solution in alcohol 1 to 2 drops under the tongue (9) and tyramine was injected subcutaneously in the subclavicular region.) The figures suggest considerable

TABLE 2

Pulse wave velocities, carotid and radial

Subject H. C. B., age 35

EXPERIMENTAL CONDITION	BLOOD PRESSURE BEFORE RECORD	BLOOD PRESSURE AFTER RECORD	PULSE RATE DURING RECORD	PULSE WAVE VELOCITY
(a) Normal		114/84	73	7.6
3 minutes after tyramine 30 mgm.	126/94	120/90	69	9.2
11 minutes after dose	120/90	110/88	62	7.2
18 minutes after dose		110/80	64	7.4
(b) Normal		109/88	62	6.7
1 minute after tyramine 60 mgm		128/100	58	9.8(?)
13 minutes after dose		112/90	61	8.5
18 minutes after dose		109/90	61	8.8
(c) Normal	107/86	104/86	56	7.5
6 minutes after tyramine 70 mgm.	107/89	115/90	67	7.1
13 minutes after dose		115/89	57	6.5
22 minutes after dose	105/79	104/79	58	7.2
(d) 8 minutes after tyramine 90 mgm.	136/98	129/88	65	7.4
11 minutes after dose	133/94	139/98	57	7.1
(e) After steady exercise for 20 minutes		110/75	132	7.6
10 minutes later	105/70	99/73	98	6.8
5 minutes after 2 drops 1 per cent nitroglycerine	83/68	81/69	110	7.6
7 minutes after dose	82/68	80/65	113	8.1
15 minutes after dose	85/65	89/68	97	9.0

variations with varying conditions and some variation even under so-called normal circumstances. If the normal figures for this subject be collected with those for normal adult males given in table 1, an average figure for the normal young adult male is found of 6.97 meters a second, agreeing therefore very closely with the figures to be found in the literature (8), (2). It seems probable therefore that the values

in young adult females are rather lower than those in the male though there seems to be no reason to suppose that this depends on anything except the differences in their normal blood pressure. The material is not sufficient to allow any positive conclusions to be drawn as to the relationship of pulse wave velocity to blood pressure, though some points as to such a relationship will be discussed later in this paper.

Apex beat to brachial. Some sample figures are given in table 3 obtained by the tambour and lever method. The average velocity is only 4.2 meters per second, very considerably slower therefore than those found by the comparison of radial and carotid. Sample records are seen in figure 1 A and 1 B, which were obtained from the same sub-

TABLE 3

Apex beat and brachial

NUMBER	SEX	AGE	BLOOD PRESSURE	PULSE RATE	WAVE VELOCITY	REMARKS
1	M.	7	90/64	139	4.1	Myocarditis and failure
2	F.	21	104/72	74	4.5	Athletic
3	F.	21	102/72	71	3.2	Athletic
4	F.	21	(a)	67	5.1	
			(b) 103/60	64	4.6	
5	F.	21	100/66	84	3.9	
6	F.	21	108/73	77	3.7	
7	M.	21	105/68	74	4.0	
8	M.	22	(a)	66	4.0	See figure 1, *a* and *b*
			(b) 113/67	66	4.1	
9	M.	24	118/77	73	4.3	
10	M.	26	108/79	60	4.7	

ject on two separate occasions (see table 3, 8). The brachial record requires no further elucidation, nor the electrocardiographic record (lead II) below, in which the high potential of the R wave is possibly accounted for by the fact that the subject was a rowing man in training. The apex beat record is quite different on the two occasions and yet by comparison with the electrocardiograph and interpreting the commencement of the expulsion period as at the point marked *E* in each case, a rising tension period of 0.05 second to 0.06 second is read in both records. The transmission rate is 4.0 to 4.1 meters a second. Supposing that the time measurements were so inaccurate as to give an error of 0.02 second too long (an error which is very unlikely considering the clear-cut character of the record and the fact that it was measured

after enlargement with a lantern), even then the velocity would not be higher than 4.8 meters a second and would therefore be considerably slower than any of the figures obtained by comparison of radial and carotid. It is to be noticed in the table how constant is the occurrence of such a slow rate in this part of the vascular system.

It can therefore be stated quite definitely that measurements of pulse wave velocity by comparison of the apex beat and the brachial show a

TABLE 4

Transmission from apex beat and radial

Subject N. B. D.

EXPERIMENTAL CONDITION	BLOOD PRESSURE BEFORE RECORD	BLOOD PRESSURE AFTER RECORD	PULSE RATE	PULSE WAVE VELOCITY	ARTERY
Normal		108/79	60	4.7	Radial
Normal		108/79	62	4.7	Radial
14 minutes after tyramine 55 mgm.	118/82	132/88	50	8.1	Radial
17 minutes after tyramine 55 mgm.	132/88	128/82	47	8.1	Radial
21 minutes after tyramine 55 mgm.	128/82	124/84	50	6.1	Radial
33 minutes after tyramine 55 mgm.	112/77	110/86	61	5.0	Radial

TABLE 5

Transmission from apex beat to brachial and radial

G. P. W., age 24

EXPERIMENTAL CONDITION	BLOOD PRESSURE BEFORE RECORD	BLOOD PRESSURE AFTER RECORD	PULSE RATE	PULSE WAVE VELOCITY		
				Apex beat to brachial	Apex beat to radial	Brachial to radial
Normal		118/77	73	4.3	4.5	4.9
13 minutes after tyramine 55 mgm.	150/76	145/74	68	4.5	5.6	8.5
17 minutes after tyramine	145/74	138/74	65	4.8	5.6	7.9
28 minutes after tyramine	118/76	122/78	75	4.2	5.1	8.2

transmission rate of only about 4 meters a second, and that this slower rate is so definitely found that it can be established regardless of any controversies as to the interpretation of apex beat records. Further figures confirming these are given in tables 5 and 6.

Apex beat to radial artery. The velocity of transmission is here also somewhat slower than that obtained by comparison of carotid and radial but faster than that from apex beat to brachial. A considerable number of figures has been obtained with a general average of about

5 meters a second and variations between 4 and 6.5 meters a second for the most part, but with considerable variations in the time under different conditions. Tables 4 and 5 give some figures obtained on two subjects before and after tyramine injection.

TABLE 6

NUMBER	AGE	BLOOD PRESSURE	PULSE RATE	PULSE WAVE VELOCITY			REMARKS
				Heart to brachial	Heart to radial	Brachial to radial	
(a) Apex beat to brachial and radial, using Frank capsules. Male subjects							
1	24	104/70	72	4.9	6.4	13.7	(See fig. 2. Normal subject though on another occasion gave higher blood pressure in leg than arm (55 mm. difference). For femoral figures see table 7, 1, and fig. 5 (taken on another day)
2	27	90/64	92	5.3	6.6	13.8	
3	28	90/64	65	3.6	4.7	10.7	Measuring from R wave and deducting 0.06 second for rising tension time
4	20	122/58	92	3.6	5.2	12.5	Aortic regurgitation. Femoral blood pressure on same occasion 190/45. For femoral figures on same day see table 7, 2, fig. 6
5	48	140/58	72	7.5	8.5	10.9	Aortic regurgitation and probably syphilitic arteritis. For femoral figures on same day see table 7, 3
(b) Figures collected by Dr. Jane Sands							
1	20	116/84	65	3.6	4.2	6.0	Heart sound record; measurements made by estimation from R wave—allowing 0.06 second for rising tension time
2	19	104/72	72	4.4	5.1	7.6	Heart sound record; measurements made by estimation from R wave—allowing 0.06 second for rising tension time
3	24	112/80	77	4.6	5.3	7.0	Heart sound record; measurements made by estimation from R wave—allowing 0.06 second for rising tension time

TABLE 6—*Concluded*

NUMBER	AGE	BLOOD PRESSURE	PULSE RATE	PULSE WAVE VELOCITY			REMARKS
				Heart to brachial	Heart to radial	Brachial to radial	
(b) Figures collected by Dr. Jane Sands—Continued							
4	22	128/94	78	4.3 3.9	5.9 5.2	14.1	See figure 3. First measurement estimated from R wave; second measurement from apex beat
5	24	128/80	100	4.8 4.3	5.9 5.1	9.0	First measurement estimated from R wave; second measurement from apex beat
6	25	128/72	65	5.3	6.0	8.6	See figure 4. Measurement from R wave as in 1
7	20	120/60	86	5.8	6.6	9.5	Measurement from R wave as in 1

Brachial artery to radial artery. It was obviously necessary to make some measurements of the transmission rate in this part of the circulatory system for comparison, and it was found, as might have been expected from the other figures, that the transmission rate was very fast. Table 5 contains some figures obtained by the lever method on one subject with records of apex beat, brachial, radial and electrocardiograph before and after tyramine. In the normal record the transmission rate from the brachial to the radial appears to be rather slow—4.9 meters a second, but as a rule much higher velocities than this have been obtained. As the transmission time over this distance was often as short as 0.02 second, the lever method with a possible error of 0.01 second was obviously far too inaccurate so that the results obtained merely showed that there was usually a very rapid velocity and nothing more.

Figures have therefore been obtained using the Frank capsules and light mirrors, again obtaining records of apex beat, brachial, radial and electrocardiograph. These have often given velocities from brachial to radial of 10 to 14 meters a second in the normal subject. One record is reproduced in figure 2. This record gave a transmission rate of about 4.9 meters a second from the apex beat to the brachial, of 6.4 meters a second from the apex beat to the radial and of about 13.7 meters a second from brachial to radial. The subject was a young healthy adult with a blood pressure of 104/70, a pulse rate of 72, and the distances

from the apex beat to the brachial and from the brachial to the radial were respectively 44 and 26 cm., the time of transmission from the apex beat to the brachial being 0.090 second and from the brachial to the radial 0.019. The rapid transmission from brachial to radial is obvious, and no experimental error could bring it anywhere near the orthodox figures of the literature. If the speed of transmission is as high as this in this region then it must be much slower in the larger vessels in order to give an average velocity of about 7 meters a second in carotid to radial measurements. Measuring from the point marked *E* on the record the transmission rates given above are obtained and though the rate to the brachial is rather high as compared with other figures given in this paper, yet the rate to the radial works out at one which is quite similar to the lower figures obtained from carotid and radial comparisons. If the time be measured from the top of the R wave to the brachial upstroke an interval of about 0.17 is obtained. The summit of the R is admitted by all to be very close to the commencement of the ventricular contraction and deducting 0.06 for the rising tension time and 0.01 second for conduction in the rubber tubes to the tambour, a transmission rate of as low as 4.4 meters is obtained for the conduction of the pulse to the brachial. So that the figures given above have probably, if there is an error, exaggerated rather than minimized the speed from the apex beat to the brachial, therefore this figure again confirms the figures already given for the brachial.

Using the Frank capsule method we have obtained on three normal subjects the figures given in table 6 (a) 1,2 and 3 and on two cases with aortic regurgitation those in the same table subjects 4 and 5 (5 probably also with arterial disease) while the figures kindly collected for us by Dr. Jane Sands are included in table 6 (b) subjects 1 to 7. Figures for the transmission rate to the femoral and dorsalis pedis were also obtained on subjects 1, 4 and 5 and will be referred to later.

Doctor Sands obtains figures which are somewhat slower in the transmission time, from brachial to radial, than those found on the few cases we have examined ourselves, but they show the same general characters of a rate much above that found in the brachial, and a rate also very variable from person to person under apparently constant conditions. Her figures give averages of 4.6 meters a second for the heart to brachial, 5.5 for the heart to radial and 8.8 for the brachial to radial, to compare with 4.2 meters a second for apex beat to brachial by the lever method and rates of 4.4 for apex beat to brachial, 5.5 for apex beat to radial and 12.7 for brachial to radial in the few cases examined using Frank

capsules and detailed in table 6 a (in arriving at these last averages the patient subject 5 has been excluded). Figures 3 and 4 reproduce two of her records (subjects 4 and 6), the former with a tambour arranged so that the apex beat pressure curves could be read and the latter with a record of heart sounds (Wiggers method).

Apex beat to carotid. Only a very few measurements of this time have been made, as the distance is short and any errors—such as, for instance, in the interpretation of the apex beat curve—would have a proportionately great effect. For this reason also we are giving no details of those measurements we have made. It is sufficient to note that we have found here a velocity of 2.5 to 4 meters a second. That a slow rate must be found was inevitable from a consideration of the other figures, since if the velocity is 8 to 12 meters a second from brachial to radial and about 4 meters a second from the heart to the brachial, a similar rate to this must be present in the carotid in order to give a rate of about 7 meters a second by comparison of carotid and radial.

Weitz (15) (1918, p. 211) in one of his tables gives figures from which the transmission time from the heart to the carotid may be obtained on twenty-three subjects. He does not give the distances from the heart, but since he used a tambour on the carotid just above the right clavicle the average distance may be taken to have been about 12 cm. and can hardly have failed to fall between 10 and 15 cm. The time of transmission varied between 0.017 second and 0.056 second with an average of 0.034 second. This average time gives a velocity of 3.5 meters a second, if the average distance be taken as 12 cm. and of 2.9 meters or 4.4 meters if the distance is taken as 10 or 15 cm. In any case the rate must be slow and of a magnitude similar to that we are putting forward.

Apex beat to femoral and dorsalis pedis. Here again up to the present only a few measurements have been made, but where measurements have been taken, figures for the velocity over both these distances have generally been obtained on the same occasion and usually on the same photograph. Determinations have been made by both the lever method and using Frank capsules. The femoral tambour has been placed on the groin and to the measurement of the distance of this point from the angle of Ludwig 10 cm. have been added to allow for the aortic arch. With these longer distances errors in measurement of distance and time are less serious.

Actual figures obtained are given in table 7 and two sample records with Frank capsules are reproduced (figs. 5 and 6). Figure 6 gives the

curves obtained from a subject with aortic regurgitation (table 7, 2) and the femoral curve shows very prettily the vibrations accompanying the systolic wave which no doubt correspond with the "pistol" sound.

TABLE 7

Apex beat to femoral and dorsalis pedis

NUMBER	AGE	BLOOD PRESSURE	PULSE RATE	WAVE VELOCITY			REMARKS
				Apex beat to femoral	Apex beat to dorsalis pedis	Femoral to dorsalis pedis	
1	24	120/70 (Arm)	72	4.5	6.4	9.0	(See fig. 5.) On this occasion a blood pressure reading was obtained in the leg (? muscular) of 175/100 (? accurate). Same subject as table 6 (a) 1.
2	20	190/45 (Leg)	87	3.6	5.6	8.3	(See fig. 6.) Aortic regurgitation. Blood pressure in arm 122/58. Same subject as as table 6 (a) 4
3	48	140/50 (Arm)	74	9.7			Aortic regurgitation with probably syphilitic arteritis. Blood pressure not readable in leg. Same subject as table 6 (a) 5
4	22	116/76 (Arm)	68	4.1			Same subject as table 3, 8 (b) and on same day
5	(a) 35	106/69 (Arm)	60	5.0	8.1	9.3 12.7	Measurements for femoral to dorsalis pedis on two separate plates
				Carotid to dorsalis pedis			
	(b)	105/75 (Arm)	64	8.5			
				Carotid to dorsalis pedis			
	(c)	108/50 (Arm)	66	9.3			

Even in the record from the dorsalis pedis these systolic vibrations are just readable, though these are not very clear on the part reproduced. Their vibration frequency on the record is about 50 per second. It is clear here again that the velocity is relativly slow over the big vessels

and much faster over the smaller peripheral ones, though the rate from the groin to the foot is possibly not so fast as from the elbow to the wrist. There may perhaps be a relatively slow velocity in the large femoral artery with a faster speed peripherally. The average velocity may be taken as from heart to femoral about 4 meters a second and from femoral to dorsalis pedis about 9 meters a second.

Discussion of results. These results indicate that variations in pulse wave velocity require further investigation taking simultaneously multiple records from different vessels in order to determine what changes in elasticity accompany different conditions of blood pressure. That the rate must be influenced by the diameter of the vessel has long been recognized, but it has usually been assumed that the slowing of the rate in vessels of large diameter would be fairly well neutralized by the quickening of the rate resulting from the thicker walls.

These considerations were reduced to a formula by Moens (12) and von Kries (11), Moens giving the formula:

$$V = c\sqrt{\frac{g\,E\,d}{s\,D}}$$

where V is the velocity of the pulse wave, c a constant, E the elasticity coefficient, d the thickness of the vessel wall, D its diameter, s the specific gravity of the fluid, and g the acceleration due to gravity.

Recently Bramwell and A. V. Hill (2) have modified these formulae somewhat and have tried to reduce to formulae the observations made in 1885 by Grunmach (8) on the effect of blood pressure on pulse wave velocity. Grunmach drew attention to the low velocities obtained in aortic valve lesions and in cases of severe anemia, and also to the high velocities obtained in cases of arteriosclerosis, while Erlanger and Hooker (6) later also noted the rapid transmission rate in this type of circulatory disturbance. Bramwell and Hill have measured the pulse wave velocity in an excised artery at different diastolic pressures, and find with an excised human carotid velocities which are very low—to their mind—in proportion to the diastolic pressures, if the usual estimates of diastolic pressure in man are accepted. Though they consider the pulse wave velocities obtained in the living person high if judged by the diastolic pressure measured by the auscultatory method, they find them to compare fairly well with those in the excised vessel, if the less generally accepted values for diastolic pressure obtained by the Paschon oscillometer are used. But in all these conclusions *they have compared the velocity in the excised carotid artery with the figures obtained*

in the living person by comparison of radial and carotid records, and it is clear from our results that this must be unwarranted. Their formulae and figures are however of great importance and it is extremely interesting to note that their excised carotid gave a velocity of 4.81 meters a second for a diastolic pressure of 78 mm. of Hg, while we have found in the living subject a velocity of from 3 to 4 meters a second from the apex beat to the carotid with a diastolic pressure of from 70 to 75 mm. It would seem as though the agreement was in reality extremely close and that their figures confirm the generally accepted auscultatory criterion for diastolic pressure, especially since it is clear that these measurements of the velocity in the carotid in the living person are only approximate figures. Again with a diastolic pressure of 58 mm. one case of aortic regurgitation we have examined gave a transmission rate of 3.6 meters as second (table 6, 4) from apex beat to brachial, while Bramwell and A. V. Hill in the excised carotid (a vessel of about the same size) find a velocity of 3.45 meters for a diastolic pressure of 57. Or again the subject for whom figures are given in tables 5 had an average diastolic pressure of 76 mm. and an average rate of transmission from the heart to the brachial of 4.45 meters a second, corresponding very well with the rate they give of 4.81 meters for 78 mm. pressure.

The question of the relationship of blood pressure to the pulse wave velocities obtained has long interested us and we have tried plotting the various results against systolic pressure, diastolic pressure and mean pressures, estimating the latter both at midway between the systolic and diastolic pressure and also nearer the diastolic pressure, taking it as equal to the diastolic pressure plus one-third of the pulse pressure. It was with the mean pressure estimated in the latter way that the figures comparing carotid and radial pulses seemed to give the best agreement, and we were inclined to assume that this was the pressure to be taken into consideration. Further investigation however of other cases of aortic regurgitation with low diastolic pressures, measurement of apex beat to brachial times, and a consideration of Bramwell and A. V. Hill's paper has led us to reconsider this, for there are few of our figures on the larger vessels which are inconsistent with those these authors have obtained on the excised carotid. Certain figures suggest that there are other important factors (compare for example subjects 1 and 2 in table 6, a) but on the whole the agreement is quite good. Figure 7 represents in a chart the results we have obtained on heart to brachial or heart to femoral measurements plotted against diastolic pressure, and on the same chart are given Bramwell and Hill's

figures for excised carotid. The agreement is quite good except for a few figures, though the range of differences in diastolic pressure is unfortunately small. The figures obtained by Doctor Sands do not agree so well and are not included in the figure. In this work the conditions could not be so carefully controlled. The pressures were taken by the students themselves with the subject standing, and the records obtained with the subject sitting.

We have found the rate of transmission along the radial very variable, more than appears to be accounted for by the increased effect of experimental errors, and it seems likely that the transmission rate in this vessel is more often affected by the local condition of the vessel as regards contraction. Note for instance the figures in table 5 and compare the changes in rate in the brachial as compared with the radial artery. Any such variability in rate involves not only a greater variability in apex beat to radial measurements, but also a similar variability in carotid to radial measurements. It is in this factor that we believe that many of our previous discrepancies have arisen. Naturally the brachial artery is also liable to constriction (anyone who has used it for arterial puncture will have been struck by the marked character of this) but it is undoubtedly less common than in the radial.

In figure 8 the readings for pulse wave velocity obtained by comparison of carotid and radial are plotted against the diastolic pressure, and comparison is again made with Bramwell and Hill's figures. There is clearly a general parallelism, though these figures all lie above those of the excised carotid. Discrepancies are rather more common, which we believe is partly explained by the hypothesis already suggested. In the figure the values obtained after nitroglycerine and given in table 2, e, are omitted, since we have made many experiments with this drug with very varying results, so that the whole question requires reinvestigation, using multiple records and Frank capsules. Some of the figures after tyramine injection also seem to be rather discordant, but variations in the conduction along the radial are probable after this drug and the blood pressure also often shows very rapid variations making the actual pressure at the time the record was taken somewhat uncertain. Apart from these the agreement is good with the exception of figures obtained in patients in whom there was evidence of arterial disease. A general parallelism was to be expected from the other results, since the time taken in transmission is expended for the most part in traversing the larger vessels.

Cases of arteriosclerosis have not yet received much investigation but in the few we describe, subjects 18, 19, 20 (and perhaps 15) of table 1 and table 7, 3, are interesting in that they have a high velocity without a high diastolic pressure, and this certainly is in favor of thickened vessels. On the other hand subjects 13 and 17 have velocities of transmission which correspond well with those to be expected in normal adults with the same diastolic pressure, if Bramwell and Hill's figures be accepted. And yet subject 17 had been under medical observation for over a year, usually showing these high arterial pressures. Similarly a subject with chronic nephritis described by Erlanger and Hooker (6) with a blood pressure of 215/130 gave a pulse wave velocity of 13.3 meters a second, which also agrees well with this curve. There is no evidence then of sclerosed vessels in the latter cases and this corresponds well with the blood pressure readings since if the other factors—arteriole resistance, heart output and pulse rate—are kept constant a sclerosis of the vessels and diminished distensibility must result in a lowering, not a raising, of the diastolic pressure. Consequently here is strong evidence confirming the distinction of hypertension patients into two classes, those with high and those with relatively low diastolic pressure, a distinction already suggested on other grounds by Stone (13). This subject is being further investigated.

One or two other figures require special mention. In table 1 subject 9 gave a comparatively normal pulse wave velocity of 6.3 meters a second by comparison of carotid and radial, though he had a blood pressure of 168/35, and this seemed to us at first to indicate that neither the systolic nor the diastolic pressure was the essential factor, but rather the mean pressure, while results such as those given in table 4 also made us doubt the validity of the use of diastolic pressure. But after making allowance for variations in the radial artery comparison with the diastolic pressure is not impossible even in table 4 and the figures given in table 5 make such agreement even probable. Again in the subject with aortic regurgitation and blood pressure of 168/35 above referred to, a velocity of 6.3 meters a second from carotid to radial would correspond to about 5 meters a second from apex beat to radial. A similar subject investigated later (table 6 (a) 4) gave a velocity of 3.6 meters a second from apex beat to brachial with a velocity of 5.2 from apex beat to radial, so that both these subjects may well have had a velocity of transmission in the brachial of about 3.6 meters a second with diastolic pressures respectively of 35 and 58. Here again the results would correspond well with the figures for the excised carotid

of 3.76 and 3.45 meters a second for pressures of 25 and 57 mm. Again it is clear that there is no disagreement between these figures and Bramwell and Hill's results, and though the evidence is not strong enough to prove that the diastolic pressure is the essential factor, it certainly does not negative this. On the other hand cases such as subject 9 of table 1 make it certain that there is no ground whatever for the comparison of pulse pressures according to the prevailing systolic pressure as suggested by Strasburger (14) nor according to the square of the systolic pressure as provisionally suggested previously by one of us (H. C. B.) (1).

How important the pressure changes may be in determining the velocity of transmission is well seen in the results obtained by Wiggers (16). He obtained simultaneous photographic records from the aorta and innominate (see fig. 3 of the above paper) and calculated the velocity of pressure propagation of the primary wave to be 3.2 meters a second—note the similarity to the values obtained by us for the larger vessels—while secondary waves at the height of the anacrotic pulse were conducted with a velocity of 17.6 meters a second, a velocity which could be reached judging by Bramwell and Hill's results at between 150 and 200 mm. Hg pressure. Unfortunately Wiggers does not give the actual pressure, but it is clear that there is a good agreement between his figures, those here collected, the figures given by Weitz for comparison of apex beat and carotid curves, and the experimental results obtained by Bramwell and A. V. Hill on an excised carotid. It seems also very probable that the last workers' results favor the correctness of the diastolic figures obtained by the auscultatory method and not those by the Paschon oscillometer as they suppose.

Out thanks are due to Dr. Jane Sands for the records and figures included in this paper and which have been already referred to in the text, and to Dr. A. B. Light who has also taken one or two records with Frank capsules for us.

CONCLUSIONS

1. The velocity of transmission of the pulse wave is much slower in the large vessels than in the smaller and more peripheral vessels. While a velocity of the pulse wave of about 7 meters a second is confirmed from a comparison of carotid and radial pulses, such a velocity is merely an average between a velocity of about 4 meters a second in the brachial and 8.5 meters a second or more between the elbow and wrist.

2. The velocity of transmission in the carotid, aorta and possibly the femoral artery is of about the same rate as that found for the brachial.

3. The rate of transmission is much more variable in the more peripheral parts of the arterial system and is in all probability much more dependent on local conditions of vasoconstriction or dilatation.

4. The rate of transmission varies with the blood pressure and in the larger vessels particularly the figures are in good agreement with those obtained on excised vessels by Bramwell and A. V. Hill. It seems probable that the results obtained on large vessels will give a much better agreement than those obtained on smaller and peripheral ones.

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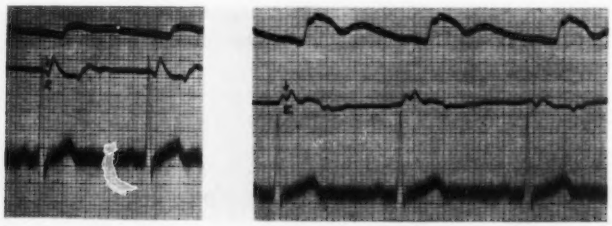

A B

Fig. 1, A and B. Reduced one-half. Records from brachial artery, apex beat and E. C. G. from above downwards on the same subject on two separate occasions. Lever method. Commencement of expulsion period marked *E*. Time intervals 0.04 second. See table 3, 8.

Fig. 2

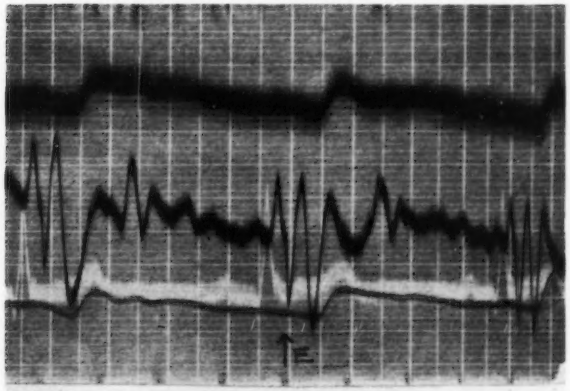

Fig. 3

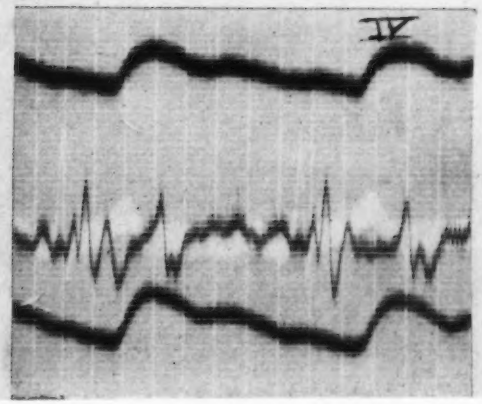

Fig. 2. No reduction. Brachial, apex beat and radial from above downwards with E. C. G. Frank capsules. Expulsion period taken to commence at *E*. All curves delayed in transmission as compared with E. C. G. by 0.010 second. Corrections for parallax require movement of radial 0.5 mm. to right and apex beat 0.5 mm. to left. Time intervals 0.1 second. See table 6, 1.

Fig. 3. No reduction. Brachial, apex beat and radial from above downwards with E. C. G. Frank capsules. Corrections etc. as for figure 3 except brachial to be moved 0.48 mm. to left and radial 0.48 mm. to right. E. C. G. is lead III—in every other record it is lead II. See table 6 (b) 4.

Fig. 4

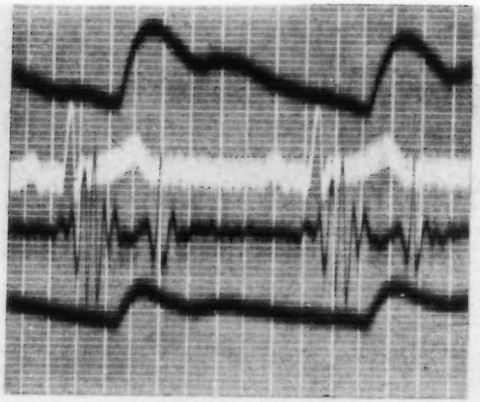

Fig. 5

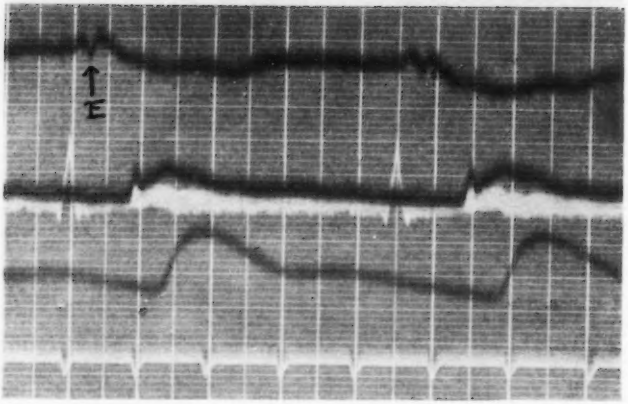

Fig. 4. No reduction. Brachial, heart sounds radial from above downwards. Otherwise as in figure 4. See table 6 (b) 6.

Fig. 5. No reduction. Apex beat, femoral and dorsalis pedis from above downwards with E. C. G. Frank capsules. Expulsion period taken as starting at *E*. Transmission time to tambours as for figure 3, but for parallax correction set apex beat 0.5 mm. to left and dorsalis pedis 0.5 mm. to right. Time intervals 0.1 and 0.2 second. See table 7, 1.

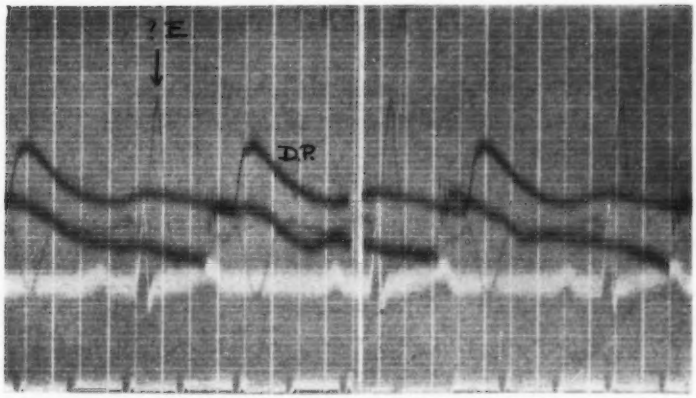

Fig. 6. No reduction. Dorsalis pedis, femoral, apex beat and E. C. G., the apex beat being the very faint record and dorsalis pedis record the one marked *D.P.* Transmission time correction as before. For parallax correction set apex beat 0.5 mm. to right and femoral 0.5 mm. to left. Time intervals 0.1 second and 0.2 second. See table 7, 2.

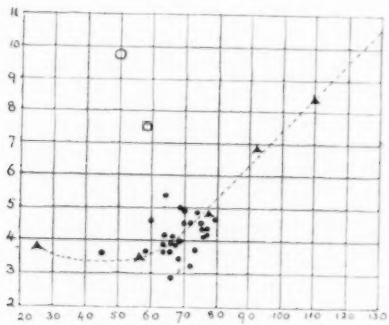

Fig. 7. Readings of heart to brachial and heart to femoral pulse wave velocities plotted against diastolic pressure for comparison with Bramwell and Hill's figures.
Abscissae—diastolic pressure by auscultatory method.
Ordinates—estimated velocity.
Bramwell and Hill's figures on excised carotid, ▲
Curve through their figures, - - - - - -
Observations on subjects with presumed normal vessels, ●
On subjects with presumed abnormal vessels, ○

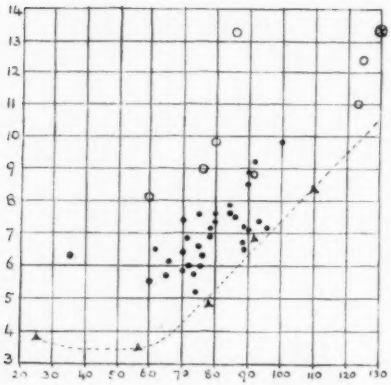

Fig. 8. Readings of velocity by comparison of carotid and radial plotted against diastolic pressure, for contrast with figure 7.

Abscissae, ordinates, etc., as in figure 7.

Value quoted by Erlanger and Hooker (8) marked ⊕

CYTOLYSIS AND PROTOPLASMIC STRUCTURE

I. Resistance Reversal Phenomena in Saponin-Hypotonic Cytolysis

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In any attempt to formulate a theory of the mechanism of cell cytolysis particular attention must be directed to the work of Rywosch (1), (2). He compared the resistance of erythrocytes of various mammals to hypotonic solutions with their resistance to saponin solutions. His results are tabulated below, the most resistant corpuscle being placed at the top with decreasing resistance grading down to the foot:

HYPOTONIC SOLUTIONS	SAPONIN SOLUTIONS
Guinea pig	Sheep
Rabbit	Ox
Swine	Swine
Ox	Rabbit
Sheep	Guinea pig

An inspection of this table brings out the peculiarly significant fact that the order of resistance to hypotonic solutions is the exact inverse of the order of saponin resistance.

The questions arise, first; to what cell elements are saponin and hypotonic resistances respectively due? Second, why do we obtain such a curious reversal of saponin-hypotonic resistance in the erythrocyte? Finally, is this reversal phenomenon exclusively found in such specialized systems as the red blood cell or is it a general characteristic of protoplasm which depends upon the relative amounts and physical state of various specific antagonistic elements? The present investigation will show this type of reversal phenomenon is exhibited by other forms of protoplasm.

Let us consider the various ways in which saponin may act on lipoids. It may simply disperse to a greater degree an already finely divided lipoid; it may also convert a water in oil lipoid emulsion system to one in which water is the continuous phase and oil the discontinuous, namely, the oil in water emulsion. This latter mode of action can be readily demonstrated by kneading lanolin with a saponin solution. At a definite concentration of saponin the emulsion is reversed, the ordinary water-immiscible lanolin emulsion being converted into a frothy, highly water-miscible lanolin in which the lipoids are now the internal or disperse phase.

Saponin also appears to cause lipoids to swell, but it is a question whether this is true imbibition or simply a dispersion of the lipoid particles. Whatever their function in the cell may be, lipoids, in particular cholesterol, play an extraordinarily important rôle in determining selective permeability and diffusion relations, especially where cytolytic agents are involved. Ransom (3) was the first to show that saponin is antagonized by certain of the lipoids, more especially cholesterol. The nature of the union between saponin and cholesterol is at present unknown though numerous authors have postulated adsorption complexes and easily dissociable compounds. Though saponin acts primarily on the lipoid fraction of the cell, it also functions by dispersing the protein moiety.

Hypotonic solutions depend for their cytolyzing action primarily on mechanical disruption produced by an inflow of water. This inflow is caused by the difference in concentrations of the substances inside the cell as compared with its environing medium. It is essentially an osmotic effect, according to the view now held most generally. It does not seem, however, that this is necessarily the true explanation.

Ova of marine Echinoderms offer exceptional advantages for study of these types of phenomena. They are obtainable in large quantities; their native medium, sea-water, is one which readily admits of chemical treatments; the viability of the eggs may be tested after the various treatments by determining their subsequent ability to divide and differentiate; and lastly, a great deal of extremely reliable data is at hand concerning the cytology and fertilization reactions of these eggs.

The eggs employed in this investigation were those of the Starfish (Asterias forbesii), the Sea urchin (Arbacia punctulata), the Sand Dollar (Echinarachnius parma) and the Annelid worm (Choetopterus pergamentaceous).

METHOD. The egg-filled ovaries were removed from the body of the animal and the eggs strained through cheesecloth into finger-bowls, avoiding admixture of the body fluid. Fresh sea-water was then added and the eggs allowed to settle. The supernatant liquid was then siphoned off and the eggs thoroughly mixed, and a 1 cc. sample removed for counting.

Counting. A 1 cc. sample was quantatively delivered into a 100 cc. volumetric flask, the flask filled to the mark with sea-water, shaken, 0.1 cc. of its contents removed and placed in a concave-bottomed watch glass for counting under the microscope,—preferably a low power binocular. After the number of eggs was determined, the necessary dilutions were calculated to make 5 cc. of the original egg suspension contain the required number of eggs.

Subsequent treatment. 1. Arbacia eggs were fertilized immediately by dilute active sperm, using finger-bowls with large quantities of fresh sea-water.

2. Sand Dollar eggs were treated in the same manner as the eggs of Arbacia.

3. Asterias eggs were allowed to maturate by standing in sea-water 40 to 60 minutes, depending upon the season and general condition of the eggs. Examination at the end of the maturation period should show complete solution of the germinal vesicle. Very dilute fresh sperm was used for fertilization.

In order that results be uniform, controls were required to exhibit at least 85 per cent development.

Eighty cubic centimeter glass stoppered bottles were used to contain 5 cc. portions of the solutions of the chemicals in sea-water. Since 5 cc. of the eggs were added to these, the solutions were made in double strengths. All the series were run with a successive decrease of one-half in the concentrations of the chemical used for treating the eggs. Three finger-bowls were arranged parallel with each solution bottle, these bowls being filled with sea-water just before being used. They should not be filled beforehand and allowed to stand exposed to the air since the constitution of sea-water rapidly changes under these conditions.

The egg suspension as prepared above was then diluted or concentrated as the case might be to the calculated number of cubic centimeters.

When all was in readiness, the temperature and time were taken, 5 cc. of eggs were delivered from a volumetric pipette into the bottle, the bottle was stoppered and shaken to insure immediate contact of

solution and eggs. At intervals of 4, 16 and 64 minutes, 2 cc. portions were removed and thoroughly mixed in a finger-bowl with fresh sea-water, the bowl being then covered with a glass plate. After 10 to 15 minutes the eggs had settled and were washed by decantation, a process which was again repeated after a lapse of 40 minutes.

Preliminary examinations were made in most cases after about 3 hours to determine whether division had started, and if that were the case, to what stage it had proceeded. After 24 hours the final examinations were made to determine *a*, the presence of "top" and "bottom" swimmers; *b*, the condition of the eggs themselves; *c*, color discharge and, finally, *d*, the degree of cytolysis. Eggs left in the solution bottles were also examined for *a*, degree of development, *b*, color discharge.

Unfertilized eggs were treated exactly as the fertilized with the exception that they were washed once and then fertilized in the finger-bowl as soon as possible after chemical treatment, the elapsed time never being allowed to exceed 15 minutes.

Hypotonic sea-water solutions were made by mixing the required amounts of tap and sea-water. The eggs were allowed to settle into the least amount of sea-water that was to be added to the solution bottle. Sea and tap-water was placed in the bottle in such proportion that adding the eggs brought the solution to the required hypotonicity. Thus, for example, if a 75-25 mixture were the most hypotonic solution used then 7.5 cc. of fresh tap-water were added to the solution bottle and 2.5 cc. of sea-water containing the required amount of eggs added. A 65-35 mixture would be 6.5 cc. fresh tap-water plus 1 cc. of sea-water in the solution bottle to which were added 2.5 cc. of sea-water containing the eggs.

In order that we might be sure that the reagents were in reality acting on the eggs and not simply coagulating the jelly that normally surrounds the eggs, parallel experiments were conducted in which the jelly was removed by the method of R. S. Lillie (4). The method consists in shaking the eggs with 0.54 M NaCl followed by gentle centrifuging.

Parallel experiments were also run using larvae one day old.

Purified Quillaja saponin obtained from Eimer & Amend Company, (New York) and digitonin from the Eli Lilly Company (Indianapolis), were employed throughout this investigation. A sample of Quillaja saponin from Mallinckrodt & Company (St. Louis) proved to be slightly more toxic then the Eimer & Amend material.

Influence of the number of eggs. In all the work close check was kept on the number of the eggs used as the following experiment will show how profoundly the results are influenced by a change in the volume of protoplasm under treatment. The work was carried out as outlined previously, varying the number of eggs from 2,000,000 to 62,000 using 0.2 per cent and 0.05 per cent Mallinckrodt & Company saponin.

This experiment demonstrates that between the limits of 62,000 and 500,000 little variation results from changes in the egg number.

It must be pointed out at the outset that the minimum lethal dose varies with the variety of saponin used and also with the resistance of the ova from year to year. However, the relative balance of saponin and of hypotonic resistances is maintained.

A consideration of the size of the egg will also show the necessity of carefully controlling the numbers used. Glaser (5) found the unfertilized Arbacia egg to have a diameter of 74.1 micra and the unfertilized Asterias egg 103.6 micra. Lillie's (6) measurements for the unfertilized Echinarachnius egg average 140 micra. Thus we have a linear dimensional ratio of 1:1.3:2, a surface relation ($4\ \pi r^2$) of 1.7:3.1:5.1 and a volume relation ($4.189r^3$) 2.1 – 5.2 – 12.9. The following convenient numbers of eggs were chosen in order to maintain fairly constant volume and surface relations, 250,000 Arbacia, 125,000 Asterias and 64,000 Echinarachnius eggs. These proportions were used throughout the work.

The following tables (numbers 1 – 6) indicate the amount of the substance just required to prevent subsequent development and are the averages of a number of determinations at each concentration. The average temperature of the water was 21.5°C. The investigation was conducted during the summers of 1920 and 1921 at the Marine Biological Laboratory, Woods Hole, Mass.

It is unnecessary to reproduce here tables showing values for digitonin, saponin and hypotonic obtained with eggs in which the investing jelly had been removed with 0.54 M NaCl. The results confirm and parallel in every respect those obtained with eggs having no NaCl treatment.

The time required for the complete cytolysis of the eggs exhibits in a most striking manner the resistance reversal phenomenon. Thus in the following experiments mature but unfertilized eggs were treated with the cytolyzing agent and the time noted for complete cytolysis of the egg. In table 7 is given the time in minutes and seconds for such cytolysis.

TABLE 1

Quillaja Saponin

TIME OF TREATMENT	OVA USED					
	Arbacia		Asterias		Echinarachnius	
	Unfertilized	Fertilized	Mature unfertilized	Fertilized	Unfertilized	Fertilized
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4	1	1	>20	>20	0.0125	0.0125
16	0 5	0.5	>20	>20	0.00625	0.00625
64	0.25	0 25	>20	>20	0.00312	>0.00312
256	No division in 0.25 per cent	No division in 0.25 per cent	>15	>15		

TABLE 2

Digitonin

TIME OF TREATMENT	OVA USED					
	Arbacia		Asterias		Echinarachnius	
	Unfertilized	Fertilized	Mature unfertilized	Fertilized	Unfertilized	Fertilized
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4	0.00625	0.00625	0.0125	0.0125	0.00078	0.00078
16	<0.00625	0.00625	>0.00625	0.00625	0.00078	0.00078
64	>0.00156	0.00312	0.00625	0.00312	0.00039	0.00039
256	No division in 0.0078 per cent	No division in 0.00156 per cent		No division in 0.00156 per cent	No division in 0.00039 per cent	No division in 0.00039 per cent

TABLE 3

Hypotonic sea-water

TIME OF TREATMENT	OVA USED					
	Arbacia		Asterias		Echinarachnius	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4	75–25	75–25	60–40	60–40	65–35	65–35
16	65–35	65–35	50–50	50–50	55–45	55–45
24	60–40	60–40	40–60	40–60	50–50	50–50

TABLE 4

Quillaja Saponin

TIME OF TREATMENT	LARVAE USED	
	Arbacia	Asterias
minutes	*per cent*	*per cent*
4	0.125	>20
16	<0.125	>20
64	0.0625	>20
256	>0.0312	>15

TABLE 5

Digitonin

TIME OF TREATMENT	LARVAE USED	
	Arbacia	Asterias
minutes	*per cent*	*per cent*
4	0.00625	0.00625
16	0.00625	0.00625
64	0.00625	0.00312
256	0.00625	0.00312

TABLE 6

Hypotonic sea-water

TIME OF TREATMENT	LARVAE USED	
	Arbacia	Asterias
minutes	*per cent*	*per cent*
4	90–10	65–35
16	>80–20	65–35
64	>80–20	65–35
256	65–35	50–50

DISCUSSION. We find in the eggs studied that the Starfish is extraordinarily resistant to saponin cytolysis whereas its resistance to hypotonic solutions is quite low. The reverse is found to hold in the Arbacia egg, namely, a high hypotonic and a low saponin resistance. The Sand Dollar egg possesses very low saponin resistance and a hypotonic resistance midway between that of Asterias and Arbacia.

Digitonin is much more highly toxic than Quillaja saponin. This is known to be true for the red blood cell as Woodward, Alsberg (7) and others have shown. The relative concentrations required to inhibit development run parallel to those of Quillaja saponin but the differences are not nearly so striking. This apparent anomaly in two such closely allied substances as saponin and digitonin will be discussed in a forthcoming paper in this series.

Tables 4, 5 and 6 show that in the case of one day larvae the saponin-hypotonic resistances are even more strikingly reversed than in the fertilized and unfertilized eggs. It has been found that the resistance of eggs will vary sometimes to a considerable degree with the temperature of the sea-water and the season but it seems to be invariably the case that if saponin resistance falls, hypotonic resistance rises, and vice versa.

Little if any difference is noted in the resistance of eggs treated before and after fertilization.

TABLE 7

Time required for complete cytolysis of mature but unfertilized ova

OVA USED	QUILLAJA SAPONIN		DIGITONIN		HYPOTONIC SEA-WATER	
	Saponin	Time of cytolysis	Digitonin	Time of cytolysis	Hypotonicity	Time of cytolysis
	per cent		*per cent*			
Asterias	20	>24 hrs.	0.05	±10 min.	70–30	1 min. 40 sec.
Arbacia	2	3 min.	0.025	5 min.	70–30	9 min.
Echinarachnius	2	1 min. 30 sec.	0.025	2 min. 40 sec.	70–30	16 min.
Choetopterus	2	50 sec.	0.025	1 min. 30 sec.	70–30	40 min.

The jelly normally surrounding the egg apparently has little to do with its resistance. The exact point of attack of saponin and hypotonic solutions will be the subject of a future communication. If we arrange a table from the results of table 7, that is, the time required for complete cytolysis of the ova, the same relations of resistances to hypotonic, saponin and digitonin are exhibited. Such a table follows in which the most resistant ovum is placed at the head of the column.

SAPONIN	DIGITONIN	HYPOTONIC
Asterias	Asterias	Choetopterus
Arbacia	Arbacia	Echinarachnius
Echinarachnius	Echinarachnius	Arbacia
Choetopterus	Choetopterus	Asterias

It is clear from the above tables that we do find the same reversal of resistance in these less highly specialized systems, the Echinoderm ova, the Annelid ova and Echinoderm larvae that Rywosch found in the red blood cell.

No attempt has been made to draw theoretical conclusions from the above results nor to speculate as to the probable reactions involved.

Dr. Robert Chambers of this laboratory has kindly criticised the manuscript.

CONCLUSIONS

1. Asterias eggs are highly Quillaja saponin resistant and have low hypotonic resistance.

2. The reverse is true for Arbacia eggs, namely, high hypotonic and low saponin resistance.

3. Echinarachnius eggs exhibit very low saponin resistance and a hypotonic resistance midway between that of Asterias and Arbacia.

4. The same relations hold for larvae of Asterias and Arbacia resistances to Quillaja saponin and hypotonic solutions, except that the hypotonic-saponin reversal differences are very much more strking than in the egg.

5. If the time required for complete cytolysis of the ova be used as an index of resistance, exactly the same resistance-reversal is found as in the living egg.

6. The jelly around the egg is not a factor in saponin, digitonin and hypotonic resistance.

7. Asterias, Arbacia and Echinarachnius all show a low digitonin resistance; however, lethal concentrations occur in the same relative proportions as found for Quillaja saponin.

8. The same reversal of resistance is found in these Echinoderm and Annelid eggs as was found by Rywosch in red blood cells of various mammals.

9. Little difference in resistance is detectable by the method used in ova treated with saponin, digitonin and hypotonic solutions before or after fertilization.

10. The inversion of a lanolin emulsion system by saponin has been demonstrated.

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THE INFLUENCE OF VARIOUS STIMULI UPON HUMAN SALIVA

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As the work of Pickerill (1) was done before the newer methods of determining reaction were available, it seemed desirable to study the influence of certain stimuli upon the reaction of the saliva in the terms of hydrogen-ion concentration, and to determine whether or not the phenomena observed by him could be measured by a more accurate method. The criticisms of determining the reaction of a complex fluid such as the saliva by titration have been voiced too frequently to require repetition in this paper, and as this was the method used by practically all workers prior to 1917, results previous to this date are open to question. In addition to the reaction of the saliva, the variations in the mucin content are of interest, as this constituent of saliva has been suggested to be a factor in the etiology of dental caries, although dental opinion is divided upon this point.

METHODS. *Collection of saliva:* Preliminary experiments in which the saliva was collected in graduated tubes by chewing paraffin, indicated that the mechanical movements of the jaws influenced the composition of the specimen as compared with samples collected without the aid of paraffin, confirming the observations of Chittenden and Richards (2) and Starr (3). Therefore, in the results presented in this paper the saliva was collected with as little movement as possible of the tongue and jaws. The quantity was limited to approximately 5 ml., which was the smallest amount with which the various tests could be made. Larger quantities concealed the effects of the different stimuli and tended to exaggerate the influence of the act of collection. The specimens were obtained at approximately the same time on each day. However, as the composition varied it was thought advisable to analyze a specimen before applying the stimulant as well as at different time-periods after, in order that the change in composition could be ascribed to the influence of the stimulant without correcting for the natural variation in the composition of the saliva. Pickerill (1) analyzed

fifty samples of saliva and computed an average which he considered as portraying the composition of normal resting saliva and differences from which as due to various stimuli. It is obvious that this was a source of error in his work.

The first specimen of saliva was collected. Then the stimulus was placed in the mouth and allowed to remain for from 1 to 2 minutes, after which it was removed by expectoration. The second specimen of saliva was collected approximately 30 seconds later and therefore usually contained some of the stimulant. The third and fourth samples were collected 30 and 60 minutes after the use of the stimulant.

Stimuli: The teeth were brushed with the following: a solution of lime water, common table salt (NaCl) and with distilled water, the last serving to show the influence of the act of brushing the teeth upon the composition of the saliva.

The following materials were chewed for 1 to 2 minutes: apple, dry bread, ice cream, orange, "sweet" chocolate, and paraffin as a control.

The mouth was rinsed with 25 ml. of solutions of citric acid (1 per cent), sodium carbonate (1 per cent), acetic acid (1 per cent), sucrose (10 per cent), sodium salt of saccharin (0.03 per cent), peppermint oil (U.S.P. "peppermint water"), oil of cloves (made similarly to U.S.P. peppermint water but half-strength), acetic acid (1 per cent) plus sucrose (20 per cent), suspension of $Ca_3(PO_4)_2$ (1 per cent), and with distilled water. The saccharin and sucrose solutions were of approximately equal sweetness.

Estimation of enzymatic action: Prinz's (4) modification of Wohlgemuth's method was used. As Sherman, Thomas and Baldwin (5) and Myers and Dellenbaugh (6) showed that the amylolytic power of diastases was most efficient in the presence of 0.05 M/1 NaCl, the starch paste was made to contain this amount when 5 ml. were mixed with 1 ml. of saliva. The amylolytic index was obtained by dividing the amount of starch paste (5 ml.) by the amount of saliva present in the tube in which no color was obtained on addition of the iodine solution. This index represents the cubic centimeters of a 1 per cent starch solution which are reduced by 1 ml. of saliva within 20 minutes at 38°C., or the grams of starch digested by 100 ml. of saliva under these conditions.

Determination of alkaline reserve, acid reserve and buffer index: The methods of Marshall (7) and of Robb, Medes et al. (8) for determining alkaline reserve were not considered suitable on account of the large amounts of saliva required. Therefore recourse was made to titration

with N/200 HCl to pH = 6.0 which gave the alkaline reserve and with N/200 NaOH to pH = 8.0, the acid reserve. The results were calculated in terms of N/1 acid and alkali per 100 ml. of saliva. The sum of the two figures was considered as the buffer index. This is the method suggested by Brown for the determination of the buffer indices of bacteriological media, except that Brown titrated to pH = 5.0 with N/20 HCl. The buffer index represents the capacity of the saliva to resist changes of reaction.

Measurement of hydrogen-ion concentration: This was determined by diluting 1 ml. of saliva with 9 ml. of "conductivity" water, adding an appropriate indicator, and comparing with the standards of Clark and Lubs. Starr (3) found that when the saliva was allowed to stand the loss of CO_2 led to an increased pH. Our specimens were tested within 5 minutes of collection and no change in pH value, perceptible by the method used, was noticed. If there was a change it was very slight and did not require correction in the present work, which is a comparison of reaction of the saliva before and after certain materials had been placed in the mouth.

Mucin determination: Following the titration with N/200 NaOH to pH = 8.0, 1 ml. of formaldehyde having this reaction was added, and the reaction was again adjusted to pH = 8.0 with N/200 NaOH. From the resulting figure, the number of grams of mucin per 1000 ml. of saliva was computed on the basis of the following data:

Mucin was precipitated from saliva by addition of 1 per cent acetic acid. The precipitate was washed with alcohol and ether and dried. Weighed quantities were dissolved in sodium hydroxide and the reaction of the solution was adjusted to pH = 8.0 with standard acid. Formaldehyde, pH = 8.0, was added and the reaction again adjusted to pH = 8.0 with $\frac{N}{200}$ NaOH. It was found that 98 mil. of $\frac{N}{200}$ NaOH were required to readjust the reaction after treating one decigram of mucin with formaldehyde. The reading obtained by the Sorenson titration was adjusted to represent grams of mucin per liter of saliva, employing this figure.

PROCEDURE. The 5 ml. of saliva were measured in 1 ml. quantities into five tubes. To four of these, 9 ml. of conductivity water were added. Bromthymol-blue was added to one tube and the hydrogen-ion concentration determined. This tube and a second tube containing brom-cresol-purple were titrated to pH = 6.0 with N/200 HCl, and then discarded. To one of the remaining tubes, phenol red was added and then titrated to pH = 8.0 with N/200 NaOH, using the second tube

as a cover tube. Formaldehyde was added and the reaction readjusted to pH = 8.0. Phenol red was added to the second tube and the process was repeated.

The last tube of the five received 1 ml. of distilled water. This was tube no. 2 in the series used for the determination of the amylolytic index. One milliliter of the contents of this tube was added to 1 ml. of

TABLE 1

Variations in the composition of the saliva of the same and different subjects on consecutive days

	pH	ALKALI RESERVE	ACID RESERVE	BUFFER INDEX	MUCIN CONTENT*	AMYLOLYTIC INDEX†
Subject A	6.8	0.79	0.56	1.35	2.80	312.5
	6.5	0.54	0.37	0.91	0.93	312.5
	6.6	0.60	0.53	1.13	2.15	312.5
	6.8	0.69	0.37	1.06	2.09	625.0
Subject B	6.9	0.72	0.29	1.01	1.59	312.5
	6.7	0.51	0.34	0.85	1.73	625.0
	6.5	0.31	0.40	0.71	1.53	312.5
	6.8	0.57	0.34	0.91	1.47	160.0
Subject C	6.8	0.72	0.29	1.01	1.82	160.0
	6.7	0.46	0.43	0.89	1.16	625.0
	6.6	0.37	0.43	0.80	1.34	160.0
	6.7	0.57	0.46	1.03	1.86	312.5
Subject D	7.1	0.84	0.19	1.03	2.25	80.0
	7.0	0.68	0.28	0.96	1.80	160.0
	6.9	0.63	0.37	1.00	1.98	160.0
	7.0	0.71	0.28	0.99	1.92	160.0

* Grams per liter of saliva.

† D 38°
$20'$

water (tube no. 3). This process was continued until the last tube no. 10, contained only 0.002 ml. saliva. The procedure of Prinz was then followed.

PRELIMINARY EXPERIMENTS. Four specimens of saliva were obtained on consecutive days from each of four subjects. The results are presented in table 1 and indicate the variability in all the constituents of saliva.

In table 2 the variations in the composition of the saliva of a single individual at different times during the day are shown. The decreases in hydrogen-ion concentration following meals were doubtless due to both the effect of chewing and to the influence of the various articles of diet. Results similar to those shown in table 2 were obtained upon repetition of the experiment. Both tables 1 and 2 indicate the error which would arise if the results of a number of determinations were averaged and departures from such an average were interpreted as due to the influence of a stimulus. For this reason each specimen of saliva was analyzed immediately before as well as after placing the stimulus in the mouth.

TABLE 2

Variations in the composition of saliva of a single subject at different time periods

TIME OF COLLECTION	pH	ALKALI RESERVE	ACID RESERVE	BUFFER INDEX	MUCIN CONTENT*	AMYLOLYTIC INDEX†
On arising	6.7	1.11	0.62	1.73	4.21	80.0
Immediately after breakfast	7.0	1.03	0.40	1.43	1.86	160.0
11:00 a.m.	7.2	0.91	0.37	1.28	1.73	160.0
Immediately after lunch	7.4	1.83	0.28	2.10	1.16	312.5
4:00 p.m.	7.1	0.74	0.40	1.14	2.17	312.5
Immediately after dinner	7.3	1.65	0.37	2.02	1.92	625.0
9:00 p.m.	7.0	0.97	0.43	1.40	3.08	625.0

* Grams per liter of saliva.

† D 38°
20′.

VARIATIONS IN THE PH. The reaction of the saliva as determined by measurement of the hydrogen-ion concentration, was changed by the process of collecting a number of specimens consecutively. Starr (3) has shown the pH of saliva may vary due to psychical stimulation. It is probable that the mental attitude of the subject had some effect upon the reaction in the results presented in table 3. For example, in the first experiment noted in this table changes in the pH are shown when no stimulus was present in the mouth.

Mastication increased the pH markedly and a slight increase remained for an hour after the chewing had ceased. Possibly, however, this last was the effect of collection. Comparatively little or no influence was exerted on the pH of saliva when an orange, an apple or a piece of dry bread were chewed. Ice cream, which perhaps cannot be said to be chewed, seemed to increase the hydrogen-ion concentration and made the

saliva slightly more acid. Sweet chocolate increased the pH to the same extent as did chewing paraffin, doubtless because both substances required vigorous mastication. The experiments with gum are interesting as this material was chewed for an hour and mastication was continued while the saliva was being collected. The pH begins to return to the original reading in spite of the action of the jaws.

TABLE 3

Influence of various substances upon the pH of saliva

SALIVARY STIMULUS	SUBJECT A				SUBJECT B				SUBJECT C				SUBJECT D			
		Time after stimulation				Time after stimulation				Time after stimulation				Time after stimulation		
	Before	30 sec.	30 min.	60 min.	Before	30 sec.	30 min.	60 min.	Before	30 sec.	30 min.	60 min.	Before	30 sec.	30 min.	60 min.
None	6.3	6.6	6.6	6.6	6.4	6.7	6.7	6.8	6.7	6.9	7.1	7.1	7.0	7.0	7.0	7.0
Chewing (paraffin)	6.7	7.1	6.8	6.8	6.5	7.1	6.7	6.7	6.9	7.2	7.1	7.1	7.0	7.4	7.1	7.1
Apple	6.6	6.8	6.6	6.8	6.7	6.8	6.7	6.7	6.8	6.7	6.9	6.7	7.0	7.2	7.2	7.2
Orange	6.8	6.5	6.7	6.9	6.7	6.7	6.7	6.7	6.7	6.6	6.7	6.7	7.0	7.1	7.0	7.0
Ice cream	6.6	6.4	6.3	6.6	6.9	6.7	6.7	6.8	6.9	6.9	6.9	6.7	7.2	7.0	7.1	7.1
Bread	6.5	6.6	6.6	6.6	7.0	6.7	6.8	6.8	7.0	7.1	6.9	6.9	7.1	7.1	7.0	7.1
Chocolate	6.5	7.0	6.6	6.6	6.8	7.0	6.8	6.8	6.7	6.9	6.9	6.8	6.9	7.0	6.9	6.9
Rinsing (water)	6.7	6.8	6.7	6.7	6.7	6.7	6.7	6.7	6.8	7.0	7.0	7.0	7.1	7.1	7.1	7.1
Citric acid	6.8	6.6	6.7	6.7	6.7	6.9	6.9	6.7	6.9	6.9	7.0	6.9	7.1	6.6	7.0	7.1
Acetic acid	6.6	6.3	6.7	6.6	6.5	6.1	6.6	6.7	6.6	6.7	6.8	6.7	6.9	6.8	7.1	7.1
Acetic acid and sucrose	6.5	5.7	6.6	6.6	6.8	5.5	6.5	6.7	6.5	6.2	6.7	7.1	6.9	6.7	6.9	7.1
Sucrose	6.7	6.5	6.6	6.7	6.9	6.7	6.7	6.8	6.7	6.6	6.9	6.9	7.0	6.9	7.1	7.2
Saccharin	6.6	6.9	6.8	6.8	6.8	6.9	6.9	6.9	6.7	7.0	7.0	7.0	6.9	7.1	7.1	7.1
Peppermint	6.5	6.9	6.9	6.7	6.5	6.7	6.7	6.7	6.8	7.0	6.9	6.9	7.0	7.2	7.1	7.1
Clove	6.4	6.7	6.6	6.6	6.7	6.7	6.8	6.7	6.7	6.9	7.0	6.9	6.9	6.9	7.0	7.1
Na_2CO_3	6.7	8.4	6.9	6.8	6.7	8.6	6.9	7.0	6.8	8.0	6.9	6.9	7.1	9.0	7.3	7.3
$Ca_3(PO_4)_2$	6.7	6.9	6.9	6.7	6.5	6.7	6.8	6.7	6.7	6.9	7.0	7.0	7.0	7.1	7.1	7.1
Brushing	6.9	7.2	7.0	6.8	6.9	6.9	7.0	7.0					7.1	7.2	7.1	7.1
NaCl	6.8	7.1	6.8	6.8	6.9	6.9	6.9	6.9	6.7	7.0	6.9	6.9	7.1	7.2	7.1	7.2
$Ca(OH)_2$	7.0	7.1	7.0	6.9	6.7	6.8	6.9	6.7	6.9	6.9	7.1	6.9	7.2	7.1	7.1	7.1

Rinsing the mouth with water changed the reaction only slightly and in half the cases produced no change at all. Citric acid depressed the pH in two cases as shown by the specimens collected 30 seconds after its use. This was undoubtedly due to its presence in the samples. Acetic acid acted similarly but later samples, 30 and 60 minutes, were 0.2 higher than the original pH readings. In two instances the acetic

acid and sucrose mixture led to a marked increase in hydrogen-ion concentration followed by a return to the original. Sucrose depressed the pH temporarily whereas saccharin increased it immediately. The essential oils, peppermint and clove, and calcium phosphate were followed by an increased pH reading. The sodium carbonate solution produced the most pronounced increase in the pH, due doubtless to the presence of this salt in the specimen. In all cases in which the hydrogen-ion concentration was decreased or the alkalinity increased, with the exception of Na_2CO_3, the degree of change was not sufficiently great to prove that it resulted from the influence of the material used as a salivary stimulant, because changes equally great were obtained in the process of collection. Brushing the teeth with salt or lime water was without any appreciable influence on the pH of the saliva, other than that exerted by the mechanical act itself.

Changes in the amylolytic index. The amylolytic index of each subject varied slightly, usually the degree of variation being one unit of Wohlegmuth's scale. Such variations as occurred were within the limits of change noted in different samples from a single subject or in specimens collected without use of the stimuli.

The process of collecting the specimens produced no change in the indices of two subjects, a decrease in the third, and an increase beginning with the 30 minute specimen in the fourth subject. Chewing was followed by an increase in all cases, in three of which the index returned to the original level after 30 minutes and in fourth remained at the new level throughout the experiment. When an apple or an orange were eaten the results were approximately the same as with tasteless paraffin. The remaining substances, ice cream, bread and chocolate, had no greater influence than could be explained as due to the act of mastication.

Rinsing the mouth with water was without marked effect. Using citric and acetic acid solutions produced an increased index in 75 per cent of the subjects and did not change the index of the remaining 25 per cent. The mixture of acetic acid and sucrose was without influence except in one case. Sucrose and clove oil did not change the amylolytic index. Sodium carbonate produced a temporary increase in two cases, no change in a third, and a decrease in the last case. Calcium phosphate gave very irregular results, its use being followed by an increased index immediately in one case and within 30 minutes in a second; no change in one subject, and a decrease after 30 seconds in the case of subject D.

Brushing the teeth led to a decrease in starch digesting power in two out of these cases but was without influence in the third subject. Brushing with lime water or salt did not disturb the amylolytic index.

VARIATIONS IN THE ALKALINE RESERVE, THE ACID RESERVE AND THE BUFFER INDEX. The alkaline reserve changes very readily as even the process of collecting the specimens of saliva was followed by an increased reserve. Mastication induced a marked increase in all cases except chewing bread, where in two of the four subjects the alkaline reserve either did not change or was depressed. Gum and chocolate chewing was followed by a large increase in the alkaline reserve. As both these substances required very vigorous movement of the jaws, the mechanical effect may be responsible for the results. In the case of gum, the prolonged chewing did not maintain the high level of the reserve for 30 minutes, either because the salivary glands were exhausted or because the material became soft and thus did not require very active mastication. The highest point of increase was immediately after the material had been placed in the mouth and then a gradual return to the initial reserve followed.

Rinsing the mouth with water either did not change the alkaline reserve or only very slightly. Citric acid increased this reserve, immediately to be followed by a decrease, whereas acetic acid depressed the reserve temporarily in three cases before an increase occurred. Sucrose and acetic acid when used together caused a very great depression of the alkaline reserve in two cases followed by an increase, while in the remaining cases the increase occurred immediately. The essential oils, saccharin and tri-calcium phosphate, produced only slight increases in alkaline reserve. Sucrose caused a depression which lasted through the course of the experiment in two cases although followed by an increase in the remaining subjects. This phenomenon was repeated when the teeth were brushed with sodium chloride despite the fact that brushing alone or with lime water produced a slight increase. Sodium carbonate, owing to its presence in the first specimens, caused an enormous increase in the alkaline reserve.

The acid reserve usually decreased in response to stimulation although only slightly. On the other hand, the return to the original level was much slower than was the case with the alkaline reserve and frequently was not reached at all during the experiment. Of the test substances chewed, bread alone caused an increased acid reserve in all the subjects. Three of the four cases responded similarly to ice cream and oranges. The act of mastication was followed by a lowering of the

acid reserve and eating an apple gave the same result. Rinsing with water or acetic acid decreased the acid reserve, while citric acid caused an increase followed by a decrease. The mixture of sucrose and acetic acid doubled the acid reserve in two cases, but only slightly increased it in the balance of the cases. This of course may have been due to the presence of some of the test substance in the specimen, as the acid reserve quickly dropped toward the original level. Peppermint and clove oils, calcium phosphate and saccharin all depressed the acid reserve. Sucrose was followed by an increased reserve in three cases. Sodium carbonate had a tendency to reduce the acid reserve after it had been eliminated from the saliva where its presence, of course, decreased the acid reserve beyond the limits chosen for the measurement of this quality. Brushing alone or with salt or lime water decreased the acid reserve slightly.

The buffer index, being the sum of the alkaline and acid reserves, is to an extent a measure of the power of the saliva to maintain its reaction. Irregularities in results were encountered more frequently than with either of the reserves, as might have been expected. The process of collecting the samples generally resulted in a buffer index progressively higher than that noted in the first specimen. Mastication was followed by a gradual decrease in one case, a decrease followed by an increase in a second, and in the remaining two cases by an increase followed by a decrease and then another increase. Eating any of the test substances led to an increased buffer index which was succeeded by a lowered index. Rinsing with water gave such irregular results that no general direction of change could be noted. Citric acid induced a slightly increased index followed by a return to the original level. Acetic acid caused a decreased index which persisted in one instance and which was followed by an increase in the other case, while in the two remaining subjects acetic acid caused an increase, followed by a decreased index. The results with a mixture of sucrose and acetic acid were very similar to those obtained with acetic acid alone. The essential oils affected the buffer index very slightly. Sucrose and saccharin reacted similarly in that their use was followed by a decrease preceding a return to the original index in half the cases, while in the remaining half the tendency was to increase very slightly. Brushing the teeth gave irregular results, each subject reacting differently. The use of salt led to a decrease and then an increase of the buffer index in two subjects; the depression of the index remained throughout the experiment in the third case; in the fourth subject there was an increased

index which remained for the course of the experiment. Lime water caused a decreased buffer index in one case, a slight increase in a second, and no change in the remaining two cases.

As the alkaline reserve was determined by the same method that Pickerill and other investigators used to measure reaction, an explanation of their conclusions as to the variability of salivary reaction is available. Pickerill noted that an increased alkalinity followed the introduction of an acid into the mouth. As the alkalinity measured by the titration method represents the same quality as has been called "acid reserve" in this paper, the two should be subject to change in the same general direction when proper stimuli are placed in the mouth. Allowing for the absence of controls made prior to each experiment and also for the greater accuracy of titration to a definite hydrogen-ion concentration as compared with titration to a color change of the indicator results comparable to those of Pickerill were obtained. Acids caused an increased acid reserve but did not materially change the hydrogen-ion concentration. The acid and alkaline reserves and the buffer index undergo change without, however, any modification of the reaction of the saliva, and this suggests that the variation of the reserves and index may be the natural method for preventing an alteration of the reaction.

VARIATIONS IN THE MUCIN CONTENT. One of the most prominent of the physical characteristics of saliva is its sliminess which is due to the mucin. Lothrop and Gies (9) have suggested that mucin may be influential in initiating dental caries although Head and several others are not in agreement with this hypothesis. It seemed worth while to investigate the influence of the test substances previously studied upon the content of this substance in the saliva.

A study of the mucin content of the saliva of the four subjects was made and, as indicated in tables 1 and 2, the mucin varied in amount with each subject daily. These observations also show that the amount of mucin is very small, being less than 0.3 per cent and occasionally less than 0.1 per cent.

The collection of the specimens reduced the amount of mucin in the saliva as shown in table 4. In all but one case this occurred during the collection of the second specimen and suggested that mucin is easily removed from the mouth. This is not surprising when one considers that the function of the mucin is to lubricate the food in preparation for its passage down the esophagus. Also none of the substances used to obtain the results shown in table 4 were desired by the subjects

TABLE 4

Variation in the mucin content of saliva in response to stimulation*

SALIVARY STIMULUS	SUBJECT A				SUBJECT B				SUBJECT C				SUBJECT D			
		Time after stimulation				Time after stimulation				Time after stimulation				Time after stimulation		
	Before	30 sec.	30 min.	60 min.	Before	30 sec.	30 min.	60 min.	Before	30 sec.	30 min.	60 min.	Before	30 sec.	30 min.	60 min.
None	2.89	1.28	1.03	0.89	1.98	1.65	1.53	1.59	1.40	1.28	1.40	1.40	1.73	1.73	1.40	1.34
Chewing	2.44	1.67	1.16	1.47	2.04	1.73	1.63	1.73	1.67	1.67	1.36	1.53	1.88	1.78	1.57	1.25
Apple	1.49	0.56	0.93	0.83	1.59	0.99	0.93	1.05	1.32	0.99	0.99	1.09	1.28	0.76	0.89	0.93
Orange	2.23	1.03	1.09	1.09	1.73	1.03	1.09	1.22	1.16	0.72	0.89	0.72	1.80	0.50	1.16	1.53
Ice cream	2.15	0.93	1.22	1.22	1.59	1.32	1.16	1.55	1.42	1.16	0.99	1.05	1.09	0.80	0.99	1.05
Bread	0.93	0.93	0.87	0.80	1.59	1.16	1.42	1.49	1.55	1.09	1.09	1.26	1.22	0.80	0.80	1.34
Chocolate	1.80	0.76	1.03	1.16	1.47	1.03	0.89	0.95	1.86	1.59	1.34	1.40	1.86	1.16	1.03	1.16
Rinsing	2.56	1.40	0.45	1.47	1.92	1.53	1.53	1.40	1.47	1.09	1.16	1.34	1.73	1.53	1.59	1.65
Citric acid	2.09	1.12	1.38	0.99	1.98	1.22	1.53	1.16	1.65	1.22	1.32	1.55	1.65	0.39	1.76	1.59
Acetic acid	1.98	0.89	1.22	0.95	1.53	0.70	1.01	1.16	1.34	1.28	1.16	1.22	1.98	1.80	1.73	1.92
Acetic acid and sucrose	2.23	0.89	1.34	1.28	1.59	1.16	1.22	1.40	1.53	1.03	1.53	1.28	1.73	1.03	1.53	1.47
Sucrose	2.75	1.09	0.89	1.09	0.89	0.70	0.89	1.22	1.28	1.16	1.28	1.09	1.47	0.89	1.47	1.47
Saccharin	2.23	0.95	1.40	1.34	1.65	1.40	1.40	1.40	1.53	1.28	1.16	1.34	1.59	1.40	1.80	1.47
Peppermint	1.80	0.80	1.03	1.28	1.03	0.89	1.28	1.16	1.16	0.95	1.28	0.80	1.92	1.28	1.80	1.47
Clove	1.86	0.80	1.16	1.16	1.47	1.34	1.28	1.47	1.47	1.40	1.34	1.28	1.22	1.03	1.65	1.65
Na_2CO_3	1.71	1.42	1.42	1.32	1.65	0.93	0.99	1.05	1.82	1.22	1.42	1.42	2.25	0.89	2.25	2.64
$Ca_3(PO_4)_2$	2.56	1.53	1.40	1.53	2.75	2.37	2.04	1.92	1.86	1.73	1.53	1.34	2.25	1.98	2.37	2.11
Brushing	3.30	1.71	1.42	1.22	1.65	1.65	1.38	1.32					2.02	1.88	1.65	1.73
NaCl	2.58	1.22	1.76	1.16	1.32	1.32	1.16	1.16	1.32	0.93	1.05	1.32	1.65	0.93	1.32	1.49
$Ca(OH)_2$	4.13	2.71	2.50	2.21	0.66	1.49	1.49	1.49	1.65	1.49	1.42	1.42	1.65	1.38	1.24	1.49

* Grams of mucin per liter of saliva.

and therefore a flow of saliva rich in mucin did not follow; food according to Pawlow being an excitant of mucin secretion. Chewing reduced the amount of mucin present in the saliva, the acid substances very slightly more so than the sweet or tasteless substances. As the acid materials, orange and apple, contain a large proportion of cellulose, this substance may have acted as a mechanical cleanser. Rinsing the mouth had the same effect as chewing, whether water or other substances were used. Acids, which have been recommended for their mouth-cleansing properties failed to eliminate any greater amount of mucin than the sucrose solution. This last was claimed by Pickerill to increase the amount of mucin in the saliva, but a reduction in the amount was observed in these experiments. Brushing the teeth gave results similar to those obtained by rinsing and chewing.

The failure of the mucin to increase in quantity consistently, eliminated any correlation between the mucin content and the acid reserve, the alkaline reserve, or the buffer index. Apparently the secretion of mucin was not affected by the factors which were responsible for changes in the other constituents of the saliva.

Gies and his collaborators (9) have advocated the use of acid mouth washes and dentifrices, basing their hypothesis upon the precipitation of mucin by acids. This very interesting theory was studied by filtering saliva and adding very dilute acids, N/200 HCl, to the clear filtrate until a faint cloud appeared. The hydrogen-ion concentration of an aliquot portion was determined and N/200 NaOH was added until the precipitate disappeared at which point the reaction was again determined. The data from these experiments are tabulated in table 5.

The pH at which the first indication of precipitation occurred varied from 4.3 to 4.9 and the average amount of N/1 HCl required was 1.229 ml. per 100 ml. of saliva. The variation in the amount of HCl was the result of different initial hydrogen-ion concentration. A very small quantity of sodium hydroxide redissolved the precipitated mucin.

Comparing the pH of saliva and the pH at which mucin begins to be precipitated, indicates the improbability of this occurring in the mouth unless a large quantity of a weak acid or a small quantity of a very strong acid were used.

Discussion. That the constituents of saliva vary in the same individual regardless of diet has been emphasized by Lothrop and Gies (10) as well as by many other investigators. From this evidence

contradictory results with stimuli placed in the mouths of different subjects might have been anticipitated as modification of the composition of saliva is dependent upon the individual both as to degree and direction of change. One subject may respond in a definite manner to a given stimulus whereas the same substance will produce the opposite effect in a second subject. Also owing to the variations which occur in the saliva of any individual throughout the day as well as daily, no norm can be established. Analysis of the saliva immediately before stimulation and comparison with results after application of a stimulus is the method least subject to error, but even here changes may be partly the result of the abnormal condition arising from the collection of the specimen.

TABLE 5

Precipitation of mucin from saliva

INITIAL pH OF SALIVA	ML.* *N* HCl REQUIRED TO PPT. MUCIN	pH AT WHICH PPT. FORMED	ML.* *N* NaOH REQUIRED TO REDISSOLVE PPT.	pH AT WHICH PPT. DISSOLVED
7.1	1.140	4.3	0.155	5.2
7.1	1.000	4.8	0.150	5.5
7.4	1.110	4.8	0.208	5.1
7.2	1.340	4.4	0.261	5.1
7.0	1.573	4.6	0.237	5.1
7.4	1.595	4.3	0.124	5.1
7.2	1.480	4.9	0.134	5.5
7.0	0.855	4.7	0.197	5.3
7.1	0.969	4.4	0.173	5.2
Ave....7.17	1.229	4.58	0.182	5.23

* Ml. per 100 ml. of saliva.

As early as 1905 Foa (11) measured the reaction of saliva in terms of hydrogen-ion concentration and found it to be $C_H = -8.2208$ in his only subject. Bloomfield and Huck (12) obtained pH readings from 6.0 to 7.3, 80 per cent of their cases having a pH of 6.6 to 7.1. Robb and his co-workers (8) observed a pH of 7.5 which was reduced to 7.0 when the CO_2 tension was increased. Starr (3) noted slightly lower pH readings than the last investigators and his results lay between the limits of Bloomfield and Huck. The hydrogen-ion concentration of the saliva of the four subjects employed in the present investigation fell within the same bounds. The slightly higher readings of Robb et al. (8) may have been due to their method of collection which was to chew paraffin, as the mechanical action of the jaws increases the pH of the saliva.

The reaction of the saliva varied very slightly under the influence of different stimuli. Alkalis, as long as they were present, altered the reaction to a greater degree than acids or neutral substances, while flavor appeared to play little or no part. This property of alkalis was noted by Bloomfield and Huck (12) who found that Dobell's solution temporarily reduced the hydrogen-ion concentration.

The variations which occurred in the alkaline index explain the "increased alkalinity" observed by earlier investigators. The increase in the inorganic elements of saliva, previously noted by Marshall (13) was responsible for differences in the results obtained by titration and would seem to be the mechanism by which the true reaction of the saliva is kept within narrow limits. The mucin content did not assist in this process, but reacted as a distinct unit, whose changes could not be correlated with those of any other constitutent of the saliva.

Gies and his co-workers have stressed the efficacy of acids as mouth-washes as a precipitation and subsequent removal of mucin seemed possible by their use. As the hydrogen-ion concentration of saliva is not varied to a degree sufficient to bring it within the range at which mucin precipitation begins, there is some question as to whether an acid mouth-wash would produce this result in vivo. Furthermore, if the apatite crystals used by Robb and his co-workers can be compared to human teeth in their resistance to destruction by acids, a hydrogen-ion concentration which will precipitate mucin will attack the teeth, for these workers found that apatite crystals in synthetic saliva having a pH = 5.0 were eroded.

SUMMARY

1. Human saliva varies in hydrogen-ion concentration, amylolytic index, alkaline and acid reserves, buffer index and mucin content both with different individuals and with the same individual at different times.

2. Mastication increases the pH temporarily regardless of the flavor of the material chewed. Substances which require vigorous chewing had more influence than soft materials.

3. The presence of alkalis in the saliva varies the pH more readily and to a greater degree than do acids or neutral substances.

4. The amylolytic index does not vary in response to stimulation under the conditions imposed by the methods described.

5. The alkaline reserve responds to stimulation readily, whereas the acid reserve is only slightly affected.

6. The mucin content of saliva is reduced readily, but this reduction is dependent to a greater degree on mechanical action than upon the taste or reaction of materials placed in the mouth.

7. The precipitation of mucin in vivo by acids may be accompanied by danger to the teeth.

8. No general conclusion can be drawn as to the response of saliva to any stimulant as the saliva of each subject was a distinct entity in its composition and in its reaction to stimulation.

In conclusion I wish to acknowledge the aid rendered by Miss Hazel Gillespie, whose assistance in the preparation of standards and checking results was of the greatest value, and also to express my appreciation of the coöperation of the members of the staff who, acting as subjects, made possible this investigation.

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THE INFLUENCE OF WATER DEPRIVATION UPON CHANGES IN BLOOD CONCENTRATION INDUCED BY EXPERIMENTAL SHOCK

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It has been repeatedly demonstrated that in conditions of shock marked concentration of the blood occurs which may directly contribute to the fatal termination so often encountered. Experimentally it has been shown (1) that a large factor of safety exists in the normal organism which allows very noticeable variations in blood concentration to take place without apparent detrimental effects. It is a matter of considerable significance, especially from the clinical viewpoint, to determine whether water deprivation to the point of producing concentration of blood has any influence in modifying the response to agents, introduced into the blood, known to cause blood concentration in normal animals. Two such substances are Witte Pepton and Histamine the influence of which in modifying blood concentration in dogs deprived of water forms the substance of the present paper.

Methods. The methods employed here were identical with those detailed in the article by Underhill and Ringer to which reference has already been made. The only modifying influence purposely introduced was the withholding of water from the experimental animals (dogs) for periods varying from five to eight days. Total solid determinations were made to check the hemoglobin estimations and, as in previous investigations in this laboratory, the two sets of figures showed a uniform correspondence although the values for hemoglobin fluctuated much more widely that did those for total solids.

PEPTONE SHOCK. In charts 1 to 4 inclusive may be found the data for changes in blood concentration under the experimental conditions outlined above. From these charts it is quite apparent that the type of curve obtained with animals deprived of water and put in peptone shock is quite different from that yielded by animals whose water supply has not been curtailed. It should be pointed out as a guide to inter-

Chart 1

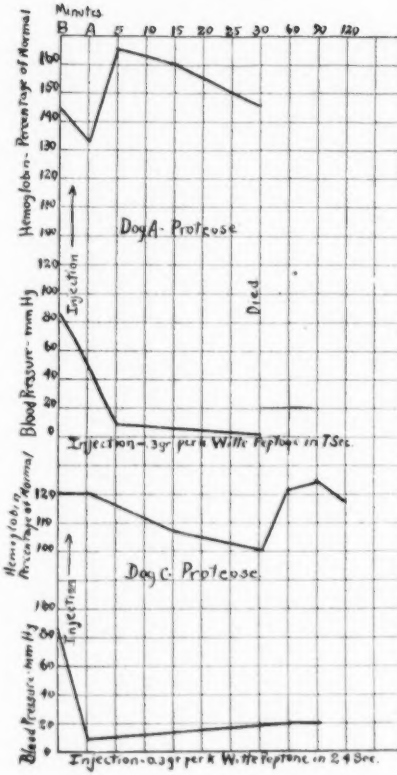

Chart 2

Chart 3

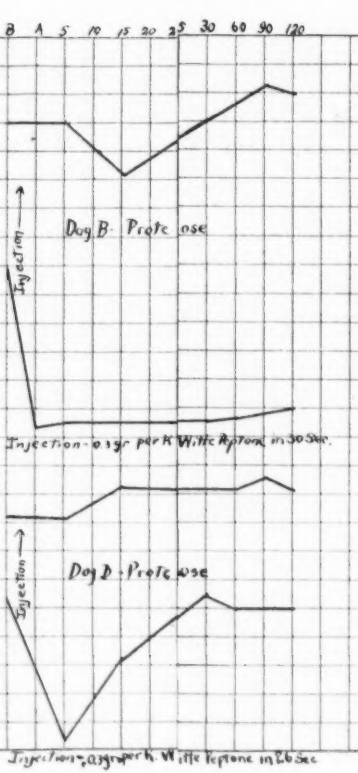

Chart 4

Chart 5

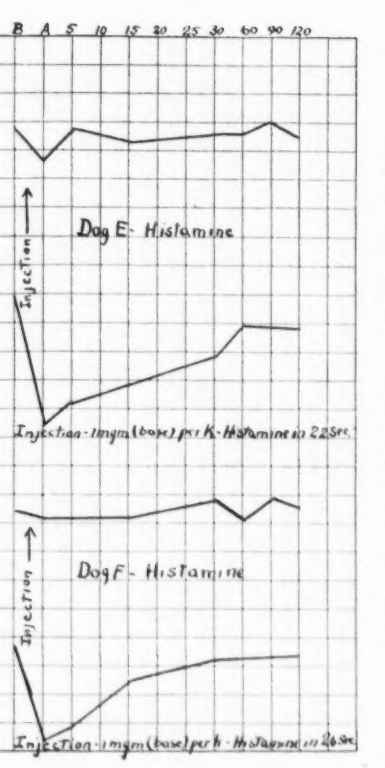

Chart 6

pretation that all these animals had blood more concentrated than normal dogs. Thus, with dog A, chart 1, blood concentration just previous to peptone introduction had reached a level 140 per cent of the initial value as a result of water deprivation for a period of 5 days. Dog B, chart 2, after water deprivation for 6 days had a blood concentration of about the same level. Dog C, chart 3, deprived of water for 8 days, showed blood concentration of 120 per cent of the initial value, and dog D, chart 4, after a similar period of water deprivation had concentrated blood to the extent of 125 per cent of the initial value.

Unlike previous experience with Witte Pepton, the almost invariable immediate effect encountered in the present investigation is the notable drop in blood concentration. This is then followed by a rise in concentration which may or may not attain a level high above the value just previous to peptone introduction. In only one case, dog A, was there a high level of blood concentration and this animal died within a comparatively short time. In how far death may have been hastened by the high blood concentration of course cannot be stated at present. In this instance a possible interpretation to account for death is the very rapid rate of peptone injection. A long experience in this type of investigation leads one to refrain from placing too much emphasis upon the fatal termination in this experiment since the fatal result of peptone injection is quite variable even in animals not deprived of water. All the other animals survived for periods sufficient to enable one to assert that the level of blood concentration attained would not have caused death. In fact, all these dogs were finally killed. While the fact that an unusually high level of blood concentration induced by Witte Pepton was accompanied by death is of considerable interest, equally worthy of note is the observation that in some animals with an already concentrated blood, injection of Witte Pepton fails to elicit the curve of blood concentration characteristic of animals whose water supply has been ample. This is true both with respect to the time of change and the level attained of blood concentration.

The response in the way of blood concentration to water deprivation is quite variable in different animals. Two animals without water for equal periods of time show entirely different levels of blood concentration and yet the reaction to Witte Pepton injection may be the same in both, that is, both may fail to show the typical rapid concentration of the blood. May not these facts be interpreted as a further proof of the large factor of safety concerned in the maintenance of water equilibrium in the body?

HISTAMINE SHOCK. With histamine injection (see charts 5 and 6) the negative result obtained is even more striking than with Witte Pepton. In animals with normal water intake even the rather slow introduction of histamine causes a rapid concentration of the blood to a distinctly high level. In the present experiments little or no increase in blood concentration is to be observed although histamine was injected very rapidly. Dog E, chart 5, had been deprived of water for a period of 8 days, attaining a blood concentration of 138 per cent of the initial value. Dog F, chart 6, with water deprivation for 8 days also had blood concentrated to a level 118 per cent of the initial value. In neither of these animals were any symptoms observed other than those usual after histamine injection into normal dogs.

SUMMARY

In dogs deprived of water for varying periods of time the intravenous introduction of Witte Pepton or histamine fails to call forth changes in blood concentration characteristically elicited in animals without water deprivation. These observations are interpreted as further proof of the large factor of safety existent in the body in the regulation of water equilibrium.

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THE INFLUENCE OF PITUITARY EXTRACTS ON THE ABSORPTION OF WATER FROM THE SMALL INTESTINE

II. Action of Pituitary Extracts when Introduced into the Alimentary Canal

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In a recent publication the author (1) showed that subcutaneous injections of pituitary extracts, in dogs and cats, caused a delay in the absorption of water from the small intestine. This was cited as a possible factor in the antidiuretic actions of these extracts.

The object of the present work was to find out whether water absorption could be as effectually limited by introducing the pituitary extracts directly into the alimentary canal as by the subcutaneous injections. This has a practical bearing on the use of pituitary extracts in controlling the polyuria of diabetes insipidus.

The author (1) has suggested that the excessive water elimination in diabetes insipidus may be due in part to abnormally excessive water absorption from the alimentary canal. The treatment of this polyuria would, therefore, be concerned in part in checking this abnormal absorption. Subcutaneous injections of pituitary extracts have been shown to be effective in controlling this polyuria, and they have also been shown to be effective in limiting water absorption. The question now arises as to whether oral administration, properly applied, would be as effective.

Up to the present time the oral administration of pituitary extracts in both experimental and clinical work has not proved to be very satisfactory, probably because of the small dosages used and on account of other improper methods of administration.

It has generally been considered that pituitary extracts are rendered inactive either by the gastric juice or by the intestinal or pancreatic

enzymes. Schäfer and Herring (2) found that peptic digestive fluid abolished the pressor action but left the "diuretic action" unaltered. They also found that reducing agents and tryptic digestive fluid leave all active constituents of the extract apparently unaffected. More recently Hamill (3) working on anesthetized or decerebrated animals found that small doses of the extract per os caused, after absorption, an increase in uterine and intestinal activity. No effect on blood pressure was noted. Diuresis was not investigated. Hamill found that pituitary extracts were soon destroyed by pancreatic juice. This, together with the fact that the above effects were obtained after ligature of the pylorus or resection of the intestine, led Hamill to conclude that the drug was absorbed in the stomach. Donaldson (4) claims to have confirmed Hamill's views clinically. He was successful in treating cases of uterine hemorrhage by oral administration of pituitary extract.

Morris and Weiss (5) were successful in the use of pituitary extracts by mouth in a case of dyspituitarism.

Rees and Olmstead (6) were able to control the polyuria in a case of diabetes insipidus by the oral administration of pituitary extracts.

Methods. Dogs and cats were used as the experimental animals. They were anesthetized with ether and kept under the anesthetic during the entire experiment.

In previous work the author (1) showed that the anesthetic was not an important factor as regards the rate of absorption or the action of the extracts. In the same article the author showed that the normal absorption rate remained practically constant during the three periods (30 minutes each) of the experiment.

The small intestine was exposed and the lumen washed, with as little trauma as possible, with warm tap water. Three ligatures were placed about the intestine, one at the pylorus, another at the ileocecal end, and the third midway between the other two ligatures. This gave two distinct absorption loops, one being the upper half and the other the lower half of the small intestine. In table 1 the upper half is designated as loop 1 and the lower half as loop 2. Glass cannulae were tied into each of the loops.

A measured amount of tap water at body temperature was introduced into each of the loops and allowed to remain there for 30 minutes, the absorption period. At the end of the 30-minute period the remaining water was removed and the amount of absorption noted.

Each experiment was divided into three periods of 30 minutes each. The first 30-minute period was taken as the control, water only being

introduced into the loops. The other two periods were used for the experimental data, water plus pituitary extract being introduced into the lumen of each loop. In eight of the experiments, designated in table 1, the pituitary extract was introduced into the lumen of the stomach by means of a hypodermic needle.

Various preparations of pituitary extract were used as indicated in table 1. Very little difference was noted in the action of these except that the desiccated substance tended to produce spasmodic contractions of the intestine which at times interfered with the experiment. Careful regulation of the dosage avoided this trouble.

The extracts were introduced into the lumen with the water or by means of a hypodermic syringe.

In about one half of the experiments simultaneous blood pressure records were taken but in no case was there any change in the blood pressure that could be attributed to the pituitary extract. In several cases it was noted that the introduction of the hypodermic needle through the wall of the stomach or of the intestine would cause a brief rise in blood pressure, but on waiting until this effect had passed off before injecting the extract no further change in blood pressure was noted.

Results and discussion. By referring to table 1 it will be noted that the rate of absorption is quite inconstant in the various animals used. In practically all cases there was, however, a marked decrease in the amount of water absorbed after the introduction of the pituitary extract. In general the retardation of absorption was as pronounced as that found after the subcutaneous injection of the extract.

In four experiments (11, 13, 15, 22) it will be noted that there was either no decrease in the amount of absorption or in a few cases there was an increase in the absorption after the introduction of the pituitary extract. It is possible that this may be accounted for by an increase of some secretion which rendered the extract inactive. What this substance may be we are as yet unable to determine. We hope to clear up this point by some work which we now have in progress.

CONCLUSIONS

Pituitary extracts when introduced directly into the lumen of the small intestine, in dogs and cats, will cause a delay in the absorption of water from the small intestine. The delayed absorption under these conditions is practically the same as that caused by subcutaneous injections of pituitary extracts.

TABLE 1

Summary of experiments on the effect of pituitary extracts on water absorption from the small intestine. Dogs were used in experiments checked with an asterisk (*), cats were used in all other experiments

NUMBER OF EXPERIMENTAL ANIMAL	AMOUNT OF WATER INJECTED INTO EACH LOOP	WATER ABSORBED						CONDITION
		First period control		Second period		Third period		
		First loop	Second loop	First loop	Second loop	First loop	Second loop	
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	
1	30	14.0	14.0	6.0	2.0			1 cc. pituitrine 0 into each loop
2	15	8.0	7.0	6.0	1.0	9.0	1.5	1 cc. pituitrine 0 into each loop
3	15	12.5	13.0	12.0	12.0	8.5	9.0	0.5 cc. pituitrine into each loop
4	15	11.5	11.0	7.5	10.0	4.0	7.5	0.5 cc. pituitrine 0 into each loop
5	20	13 0	11.0	4.5	6.0	4.0	0.0	1 cc. pituitrine 0 into stomach
6	20	9.0	10.0	6.0	7.0	5.0	2.5	1 cc. pituitrine 0 into stomach
7	15	7.0	10 0	4.0	7.0	4.0	2.0	0.5 cc. pituitrine 0 into each loop
8	15	7.0	10.5	2.5	1.0	0.0	0.5	0.5 cc. pituitrine 0 into each loop
9	20	16.0	10.0	9.5	4 5	9.5	6.0	0.5 cc. pituitrine 0 into stomach
10	20	11.5	9.5	4.5	0.5	1.0	0.0	0.5 cc. pituitrine 0 into each loop
11	20	18.0	10.0	18.0	11.0	17.0	7.0	1 cc. pituitrine 0 into stomach
12	20	9.5	10.0	8.0	8.5	8.5	10.0	0.5 cc. pituitrine 0 into stomach
13	15	10.0	10.5	8.0	11.0	9.0	11.5	0.5 cc. pituitrine 0 into stomach
14	20	18.5	13.0	14.0	9.5	13.0	7.0	0.5 cc. pituitrine 0 into each loop
15	25	17.0	17.0	12.0	16.0	11.5	15.5	1 cc. pituitrine 0 into each loop
16*	75	53.0	57.0	32.0	33.0	22.0	17.0	1 cc. pituitary extract (Lilly) in each loop
17*	70	40 0	49 0	29.0	24.0	17.0	23.0	1 cc. pituitary extract (Lilly) in each loop
18	15	7.0	7.0	1.5	4.0	4.0	3.5	0.2 gm. pituitary body (Armour's) in each loop
19*	100	65.0	70.0	24.0	32.0	4.0	19.0	1 cc. pituitary extract (Lilly) into stomach
20*	150	112.0		65.0		76.0		1 cc. pituitrine 0 into stomach
	100		81.0		65.0		55.0	
21*	100	74.0	88.0	57.0	56.0	27.0	19.0	0.2 gm. pituitary body (Armour's) into each loop
22	20	10.0	15.0	10.0	8.0	8.5	8.0	1 cc. pituitary extract (Lilly) in stomach
23	20	16.0	19.0	11 0	13 0	16.0	17.0	1 cc. pituitary extract (Lilly) in each loop

The injection of pituitary extract into the lumen of the stomach will cause a delay in water absorption from the small intestine. Pituitary extract introduced directly into the lumen of the small intestine or stomach does not affect the blood pressure.

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STUDIES OF THE THYROID APPARATUS

VIII. On the Alleged Exogenous Source of the Poisons Giving Rise to Tetania Parathyreopriva

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There are to be found scattered through the literature on the parathyroid glands incidental observations to the effect that a meat diet after parathyroidectomy is more conducive to tetania parathyreopriva than is a diet lacking in this ingredient.

Quite recently Luckhardt and Rosenbloom (1), (2) have reported that they have been able to control and cure parathyroid tetany in dogs by intravenous injections of Ringer's solution and by preventing constipation, when meat is fed. This is a valuable contribution to the subject, both from the theoretical and from the practical point of view. It demonstrates that the tetany is due to poisonous substances within the organism which can be eliminated by appropriate treatment. It also points to a possible means of controlling the tetanies arising from sources other than a parathyroid deficiency.

However, the limited conclusions drawn by these investigators, that "*the*[1] cause of the tetany is an exogenous poison or poisons derived chiefly from the proteins (more especially the meat) of the food" and "*The* source of the poison responsible for the tetany is of exogenous origin—"are not supported by the existing data, if the customary interpretation of the terms *endogenous* and *exogenous* are employed.

That a high protein, especially meat, diet may be *a* factor in producing exacerbations of tetanic manifestations is quite probable; that it is *the* factor is not so, as the experiments reported here will show.

That it may be a direct factor is evident from the fact that meat contains small amounts of creatine and other possible precursors of a tetany-producing compound-methyl-guanidine found to be produced in increased amounts during parathyroid and other tetanies (3), (4). That it may be an indirect factor is evident because the extractives and

[1] Italics mine.

products of its digestion when absorbed tend to increase the irritability of the nervous system. This increased irritability means an increased susceptibility to the poisons already derived from the perverted metabolism arising from the loss of the parathyroid secretion. It is most probably a combination of these processes which gives rise to the increased tetany after meat feeding.

The writer has already published two reports (5), (6) of observations on the mortality of three groups of rats following parathyroidectomy due to acute tetany. The diet of all the groups was the same and contained meat (beef). Yet but 13 per cent of one group died within 48 hours after parathyroidectomy, while 79 and 92 per cent of the other two groups succumbed during the same period. This is direct evidence of the participation of an endogenous factor in the susceptibility of the rat to death from parathyroid tetany. Otherwise, if diet alone was the cause, the mortality rate in the three groups should have been the same. The interpretation of these differences was founded on the idea of differences in the endogenous production of toxic substances following parathyroidectomy, because of the observed differences in muscle tone and neural stability of the three groups studied.

These observations, however, are not completely satisfactory evidence for the point in dispute. In order to demonstrate that there is an endogenous source of the poison responsible for parathyroid tetany, the following tests were made.

Fifty tame, but not particularly gentle albino rats, ranging in age from 50 to 80 days, were divided into two groups and put into separate cages which contained no bedding. After a preliminary fast of 24 hours, there was given to one group containing 10 females and 14 males, a diet of fresh, lean, raw beef only, every day for 4 days. To the other group, consisting of 11 females and 15 males, there was given a diet of fresh lettuce only, for a similar period. Both water and food were supplied *ad lib.* Thus one group of rats was ingesting a diet almost exclusively protein and high in extractives, while the other group was subsisting on a diet containing but little protein or extractives. After 4 days all the rats were parathyroidectomized. Two of the rats of the lettuce group died under ether. There were thus left for observation 24 rats in each group. The diet of both groups after parathyroidectomy was the same as during the preliminary period; meat for the meat group, lettuce for the lettuce group. As in the earlier studies, the number of deaths occurring within 48 hours after parathyroidectomy was taken as the standard of comparison. The rats that died, died in acute tetany.

Of the 24 parathyroidectomized rats on the meat diet, 9, 5 males and 4 females, or 37.5 per cent, died within the stated period. Of the 24 parathyroidectomized rats on the lettuce diet, 14, 8 males and 6 females, or 58.4 per cent, died within the same period.

In the accompanying table are given the statistical data with regard to the body weight of the two groups. It is seen that at the beginning of the period of feeding no valid difference existed between the two groups. It is to be noted that the rats on the lettuce diet lost more weight than did those on the meat diet. This fact is indicative of a more intense catabolism in the former.

On the basis of the observations of Luckhardt and Rosenbloom (2) it is justifiable to assume that if *the* source of the poisons of parathyroid tetany is exogenous, and particularly derived from the meat of the diet, there should occur a higher mortality rate in parathyroidectomized rats

TABLE 1

Giving the statistical data of the body weight of the two groups

	MEAT DIET		LETTUCE DIET	
	Beginning	At operation	Beginning	At operation
Mean	87.0	84.1	83.7	65.7
Stand. dev.	21.8	19.7	13.6	11.9
P. E. M.	3.0	2.7	1.8	1.6

P. E. M., Probable error of mean.

fed meat, than in rats fed a diet of insignificant protein content and no meat. Our study did not yield results supporting this assumption.

On the contrary those rats which were fed a diet containing only 0.17 per cent (7) of protein and which from their 22 per cent loss of body weight were evidently in a state of rapid catabolism, were the more susceptible to the loss of the parathyroid secretion, than were the rats which were fed a diet containing some 17 per cent of protein in the form of meat, and which lost only 3 per cent of their original weight.

In the one group there was a low exogenous metabolism of protein and a diet yielding but few calories, accompanied by a relatively high endogenous catabolism producing relatively large amounts of the products of the dissolution of the body tissues, and in this group there was a high mortality rate from parathyroid tetany. In the other group, on the meat diet, there was a high exogenous protein metabolism and a diet yielding more calories, accompanied by but relatively little endogenous catabolism (8) and therefore but little of the products of

tissue disintegration, and in this group there was a low mortality rate from parathyroid tetany.[2]

These findings, therefore, disprove the conclusion of Luckhardt and Rosenbloom (2) that "*The* source of the poison responsible for the tetany is of exogenous origin (particularly the meat of the diet)," as far as the albino rat is concerned, and I see no reason why they are not applicable to other omniverous mammals.

The favorable effects of the continued treatment used by the above investigators on their thyro-parathyroidectomized dogs are probably, in part, due to the fact that metabolic disturbances, such as defects in calcification of the teeth, in chronic mild parathyroid tetany, are less marked in rats after thyro-parathyroidectomy, than after parathyroidectomy alone. This phenomenon is discussed in a forthcoming article (9).

CONCLUSIONS

These studies yield evidence justifying the conclusion that endogenous metabolism, particularly the catabolic phase, is a source of the poisons giving rise to tetania parathyreopriva, and that the statement of Luckhardt and Rosenbloom that "*The* source of the poisons responsible for tetany is of exogenous origin (particularly the meat of the diet)," fails to express the actual state of affairs.

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[2] It may be added that 72 hours after parathyroidectomy the mortality rate for the rats of the meat group was still 37.5 per cent while that of the lettuce group had risen to 75 per cent.

THE EFFECTS ON THE CIRCULATION AND RESPIRATION OF AN INCREASE IN THE CARBON DIOXIDE CONTENT OF THE BLOOD IN MAN

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The purpose of the experiments recorded here was to establish the normal curves of the circulatory and respiratory responses to a gradual increase of carbon dioxide in the inspired air and to determine whether there is an after-effect.

A gradual increase of carbon dioxide in the inspired air was secured by allowing the subject of experimentation to rebreathe 52 liters of air in a Larsen-Henderson rebreathing apparatus (23). In one series of 52 experiments, preliminary to the rebreathing, oxygen was added to the atmospheric air in sufficient amount to raise the content to about 30 per cent and then throughout the period of rebreathing a small flow of washed oxygen was allowed continually to enter the reservoir of the apparatus. In another series of 20 experiments the subject rebreathed the 52 liters of atmospheric air with the result that carbon dioxide not only accumulated but the oxygen content of the air was gradually exhausted. Preceding each experiment preliminary observations were made on each physiological condition to establish the normals for a basis of comparison; and during the experiments the respiratory changes, the pulse rate and arterial blood pressures were recorded. Determinations of the composition of the alveolar air, venous and capillary blood pressures, the hand volume and the rate of blood flow through the hand were added as the occasion permitted and included 8 to 10 studies of each.

The respiratory data were obtained by recording the movements of the spirometer drum on a kymograph and also by reading the minute volume of breathing with the Larsen automatic recorder (23). The alveolar air samples were taken from a special opening in the mouthpiece by the method of Haldane and Priestley (9). The arterial blood pressures were determined with the Tycos sphygmomanometer by the

auscultatory method; the venous blood pressure with Hooker's glass capsule; the capillary blood pressure with the Danzer-Hooker "micro-capillary tonometer;" the hand volume changes with the arm plethysmograph and the blood flow through the hand by two methods, Stewart's (26) hand calorimeter and Hewlett's (13) cuff device.

There were 16 subjects who served for a total of 72 experiments. The time of rebreathing lasted from 17 to 32 minutes with an average of 23.5 minutes; the percentage of the final content of carbon dioxide ranged from 5.7 to 9.3, mean 7.3 $\pm$ .075.

A gradual increase in carbon dioxide up to the percentages experienced in this study does not as a rule cause disagreeable sensations. There may be a considerable change in the breathing before the hyperpnea is noticed; but when the carbon dioxide has risen to 4 or 5 per cent, it is no longer unnoticed and more and more, as the carbon dioxide increases, does the air breathed fail to satisfy the intense longing for fresh air; furthermore the effort to fill and empty the lungs reminds us of the respiratory urge of strenuous physical exercise. After a while there is a feeling that the inspiratory efforts begin before expiration is completed.

Some persons develop a headache at about 5 per cent CO_2 which may be intense, but it has not been observed to last for more than 20 minutes after the experiment. An occasional person felt slightly dizzy when the breathing was greatest and a feeling of nausea has occurred. One subject, in several trials, complained of pains in the chest and expectorated some brown colored sputum streaked with blood. This subject had the highest per minute volume of ventilation obtained at 8 per cent CO_2. There has been no evidence that the man suffered any lasting ill effects from these experiences.

THE CIRCULATION. *Pulse rate.* Our data clearly give evidence that the heart rate is accelerated by carbon dioxide. In only 3 out of 72 experiments was the rate unaffected. For the group of experiments in which a high percentage of oxygen was maintained in the inspired air the mean pulse rate for each per cent of carbon dioxide up to 6 per cent has been plotted in figure 1 and tabulated in table 1. Because the number of cases at 7 per cent carbon dioxide was somewhat reduced and at 8 per cent much reduced, we have added the average increases for these percentages to the mean pulse rate of 6 per cent carbon dioxide instead of calculating the mean rate. The curve of the means indicates that the pulse rate is slightly increased even at 1 per cent, but the stimulating action is most pronounced from 5 per cent and upward.

The group of 20 experiments, in which the oxygen content of the inspired air decreased as the carbon dioxide increased, had a mean normal pulse rate of 71.3 beats. The mean pulse rate for each percentage of carbon dioxide from 1 to 7 per cent was as follows: 72.6, 73.1, 73.5, 75.6, 76.5, 78.5 and 82.9 respectively. The total increase for this group up to 7 per cent was 11.6 beats, and for the group in which the oxygen was maintained at about 30 per cent was 15.5. This is a difference that cannot be attributed to the available oxygen, but is probably explained by the fact that in the high oxygen group we were dealing with individuals who had normally a more rapid and apparently a more responsive heart; their mean normal rate was 78.5 as against 71.3 for the other

TABLE 1

Mean pulse rate and arterial pressures for 46 experiments

	PULSE RATE	ARTERIAL PRESSURE IN MM. Hg.		
		Systolic	Diastolic	Pulse
Normal	78.5	106.1	71.3	35.4
1 per cent CO_2	79.8	109.0	73.8	36.1
2 per cent CO_2	80.7	109.5	74.3	36.6
3 per cent CO_2	82.8	111.3	75.1	37.2
4 per cent CO_2	84.4	113.5	77.3	37.6
5 per cent CO_2	86.0	116.7	79.1	37.7
6 per cent CO_2	90.0	122.5	83.0	40.1
7 per cent CO_2	94.0	127.4	84.6	42.8
8 per cent CO_2	97.3	134.1	91.1	43.0
Last on	93.6	128.7	83.6	45.5
First off	90.4	115.7	71.7	46.6

group. In both groups of experiments there is evidence of a slight stimulation at 1 and 2 per cent of carbon dioxide, with a stronger action from 5 per cent and onward.

Some subjects were much more sensitive than others. One resistant man tested on six consecutive days always went to between 6 and 7 per cent carbon dioxide before there was any evidence of stimulation. Another subject in four trials always began to respond around 4 or 5 per cent, while 3 individuals always gave the first evidences of response around 3 and 4 per cent. One man tested on six consecutive days became more sensitive as the days passed, on the first two days the pulse rate first began to accelerate at 6 and 7 per cent of carbon dioxide and on the last days at 1 and 2 per cent. Several others showed a considerable variability. On the whole it can be said that the individual shows a

disposition to respond at about the same percentage of carbon dioxide in several exposures. So the pulse rate curve of figure 1 represents a generalized condition rather than an individual curve of response.

While the after-effect on the pulse rate of breathing carbon dioxide has not always been watched, yet our observations, which were often continued for 5 to 7 minutes after the subject was restored to atmospheric air, show that the effect persists for some time. In nearly every case the pulse rate had not returned to normal in the post-period of

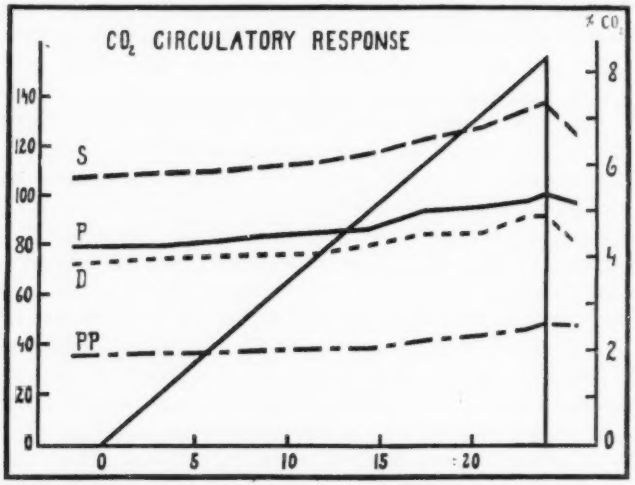

Fig. 1. The mean circulatory response of 48 cases to a gradual increase in inspired CO_2. *P*, pulse rate; *S*, systolic pressure; *D*, diastolic pressure; and *PP*, pulse pressure.

observation. The mean pulse rate for 46 experiments was 93.6 at the last count just before the subjects were restored to air, and still 90.4 one minute later.

THE ARTERIAL PRESSURE. *Systolic arterial pressure.* In table 1 and figure 1 are given the mean results of the carbon dioxide influence on the systolic pressure in experiments in which the oxygen content was greater than that of atmospheric air. The normal pressure was 106.1 mm. and the means for 1 and 2 per cent of carbon dioxide were 109 and 109.5 mm. This slight rise was not the effect of carbon dioxide, as the readings for the first 2 minutes will show, but is a psychic rise such as is

commonly present in experiments of this type. It appears, therefore, that carbon dioxide is without influence on the systolic pressure until it has increased to between 2 and 3 per cent in the inspired air. As the content of inhaled carbon dioxide continues to increase the systolic pressure rises in ever increasing degree.

The group of 20 experiments, in which the available oxygen was gradually decreased throughout the period of rebreathing, had a mean normal systolic pressure of 108.8 mm. The mean pressure for each percentage of carbon dioxide from 1 to 7 per cent was as follows: 108.6, 109.4, 112, 113.8, 115.6, 119.4 and 123.3 mm. The effect upon the systolic pressure was somewhat greater in those cases in which the available oxygen was higher than in atmospheric air. The onset of the action of carbon dioxide was, however, about the same for both groups.

While individual differences in the time of response were present there was, however, greater constancy in the percentage at which the rise began than occurred with the pulse rate acceleration. In the majority of experiments the systolic pressure began to rise at 3 or 4 per cent of carbon dioxide, in only 4 did the rise appear as late as at 6 or 7 per cent. In two of the experiments no change occurred, but these individuals reacted at other times with the ordinary type of response.

The after-effects on the systolic pressure were less persistent than on the pulse rate. There was a rapid fall in pressure during the first minute after the subject was restored to atmospheric air, this being followed by a period of more gradual decline. The mean pressure for the 46 experiments just prior to restoring the subjects to atmospheric air was 128.7 mm. and 1 minute after receiving the air was 115.7 mm. The pressure was often back to normal in 2 or 3 minutes; but sometimes the final return to normal, following the sudden drop, was delayed during the entire post-period of observation which was continued for from 5 to 7 minutes.

Diastolic pressure. The diastolic pressure increased in both types of experiments. In a considerable number of cases this pressure started to rise before the systolic but often the two pressures rose together, the systolic rising most rapidly; but in all cases the diastolic pressure continued to augment as long as the carbon dioxide increased in quantity. That the diastolic is as a rule affected earlier than the systolic pressure is shown in the curve of the means in figure 1. The total average rise when 7 per cent carbon dioxide had been reached was 13.3 mm. for the high oxygen and 7.7 mm. for the low oxygen group. So in the diastolic response, as in the systolic, the high oxygen group appeared to be most sensitive to the carbon dioxide.

The after-effects fall into two classes. In about 75 per cent of the experiments the diastolic pressure went slightly subnormal, or at least returned to normal, within the first minute after atmospheric air was given; while in the other 25 per cent this pressure remained above normal for the entire period of 5 to 7 minutes of post-observation. For the high oxygen group the mean pressure for the last reading when under the influence of carbon dioxide was 83.6 mm., and 71.7 mm. 1 minute after air was given. The mean for the preliminary normal was 71.3 mm.

Pulse pressure. Since the systolic rose more than the diastolic pressure, there occurred an increase in the pulse pressure which is first clearly defined at between 5 and 6 per cent of carbon dioxide. The mean pulse pressures at intervals up to 8 per cent carbon dioxide have been plotted in figure 1.

VENOUS BLOOD PRESSURE. The influence on venous pressure of a gradual increase of carbon dioxide in the inspired air was determined on 11 persons. A summary of the data obtained from the high oxygen group is given in table 2. In 21 experiments conducted under the two conditions the pressure, with only two exceptions, rose steadily during the period of rebreathing. The average venous pressure in the high oxygen group prior to the rebreathing was 5.4 cm. H_2O. By averaging the cases up to 5 per cent carbon dioxide and then adding to the 5 per cent result the average increase for 6 and 7 per cent carbon dioxide, because of the dropping out of cases at the higher percentages, the following curve of change in the venous pressure was obtained: 6.1, 6.7, 7.7, 7.8, 8.3, 8.8, and 9.5 cm. H_2O. So at 7 per cent of carbon dioxide the venous pressure showed an average increase of 74 per cent. The average change throughout the experiments is plotted in figure 2. The stimulating action was usually already evident at 1 per cent carbon dioxide, but in some became more effective at between 4 and 5 per cent.

The majority of observations were made on the low oxygen group, six of these with the Hooker method and the remainder with the Henderson method, in which the height of the hydrostatic column was recorded in the veins of the arm. However, in these experiments the rise in pressure was easily seen and averaged 3.9 cm. of H_2O.

The after effect on the venous pressure was about as prolonged as on the pulse rate. In 4 of the high oxygen cases the pressure was still above normal 5 to 7 minutes after the subjects were restored to atmospheric air. The other 3 cases came back to normal in 1, 2 and 4 minutes, respectively. In the low oxygen group in 9 out of 14 experiments the venous pressure was still well up 5 to 10 minutes after the rebreathing period.

CAPILLARY PRESSURE. Our capillary pressures were not corrected for hydrostatic pressure. Since only the change in pressure was of interest to us, the subject was required not to move hand and body during the experiment. We have omitted the correction because Danzer and Hooker (5) found it to be of uncertain value. The capillary pressure determinations are given in table 3. In each case they were made

TABLE 2

Venous pressure in cm. H_2O

	NORMAL	PER CENT OF CARBON DIOXIDE							LAST ON	FIRST OFF
		1	2	3	4	5	6	7		
G. C.	1.0	2.3	2.5	2.3	3.0	3.5	3.9		3.9	1.7
E. C. S.	11.3	11.4	11.8	11.4	11.8	11.9	13.0	13.6	15.2	12.2
D. T.	4.3	4.5	5.4	9.3	9.3	9.8	10.0	10.5	10.5	8.1
L. E. T.	1.6	4.1	4.6	5.9	6.3	7.2	6.8	6.7	7.0	7.4
K. O. N.	4.8	4.8	5.0	5.0	5.1	5.6	6.4		7.0	7.6
I. F. P.	14.0	14.0	14.8	16.2	15.6	16.4			18.8	17.2
C. J. B.	0.7	1.5	3.0	3.8	3.8	3.4	4.3	5.9	7.6	0.2

TABLE 3

Capillary pressure in mm. Hg

	NORMAL	PER CENT OF CARBON DIOXIDE								LAST ON	FIRST OFF
		1	2	3	4	5	6	7	8		
E. C. S. 1	38	36	52	63	67	72	73	62		60	48
E. C. S. 2	24	24	24	24	25	28	34	35	36	36	24
E. C. S. 3	29	29	32	35	37	37	39	43	49	49	34
D. T. 1	33	39	41	42	43	45	47	52		59	50
D. T. 2	40	37	38	35	42	44	46	49		49	38
D. T. 3	28	30	29	27	25	30	33	42	46	46	33
L. H. B.	41	44	46	58	54						
C. J. B.	10	14	15	15	15	15	17			17	15

on a single vessel and each record is the average of several readings which were made at regular intervals throughout the experiment. A definite rise occurred in each experiment; this appeared in 3 cases as early as 1 per cent carbon dioxide, in 2 at 2 per cent, in 2 at 4 per cent, and in 1 not until 5 per cent.

The curve of change is given in figure 2. The average capillary pressure before the exposure to carbon dioxide was 30.4 mm. Hg, this was increased as follows at the respective percentages of carbon dioxide

from 1 to 7: 31.6, 33.4, 37.4, 38.5, 40.9, 43.5 and 45.7 mm.; the last three, because cases were dropping out, are not averages but were obtained by adding the average increase for the given per cent to the preceding number. So at 7 per cent carbon dioxide the average capillary pressure was 50.3 per cent above normal.

When a subject was restored to atmospheric air the capillary pressure returned rather quickly to normal. The average pressure was 45.1 mm. for the last determination of the carbon dioxide effect and was 34.6

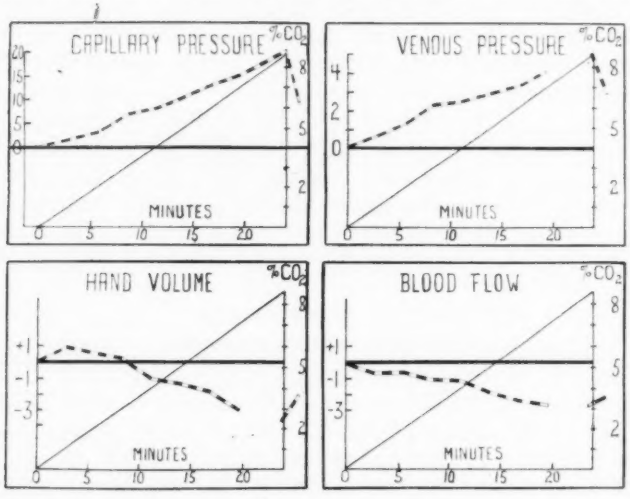

Fig. 2. Curves of the mean response to a gradual increase of CO_2. Normals have been recorded as O, the curves show the amount of change above and below normal. The scale to the left is in mm. Hg for capillary pressure, cm. H_2O for venous pressure, cc. for hand volume, and grams of blood per 100 cc. hand volume for blood flow.

mm. 1 minute after being restored to air, while the further return to normal was made within the next 3 or 4 minutes. In the rate of return to normal the capillary pressure reacted similarly to that of the arterial pressures and did not correspond to the slow return of the pulse rate and the venous pressure.

Hand volume. The changes in hand volume were followed in 8 persons by using a plethysmograph in which the hand was surrounded by water heated to body temperature. The changes in volume were read directly on a graduated tube connected with the plethysmograph.

The entire apparatus was suspended by a coiled steel spring so that the movements of respiration did not cause a thrust and suction action of the hand into and out of the chamber of the plethysmograph. The subject was instructed to sit quietly. The usual changes in the hand volume are given by a curve in figure 2. Seven out of eight subjects gave the typical response in which a slight increase in volume occurred during exposure to from 1 to 3 per cent of carbon dioxide, following this the volume slowly returned to normal and went subnormal at 4 or 5 per cent carbon dioxide. The increase in volume above normal reached as much as 4 cc., while the greatest decrease below normal was 7 cc.

The exceptional case had a steady increase in hand volume throughout the entire experiment. Along with this increase in hand volume the systolic pressure, during the first half of the experiment, gradually went

TABLE 4

Blood flow in grams per 100 cc. of hand volume

	NORMAL	PER CENT OF CARBON DIOXIDE							POST PERIOD
		1	2	3	4	5	6	7	
E. C. S.	2.6	2.3	2.0	2.2	2.6	2.2	2.2	2.1	3.3
D. T.	10.5	7.6	7.4	6.8	5.6	4.6	4.0	4.0	2.4
G. C.	0.9	0.8	0.8	0.5	0.4	0.4	0.6	0.8	0.6
L. E. T.	6.5	6.5	7.9	6.8	6.8	5.9	5.0	4.2	6.3
K. O. N.	12.7	12.6	10.0	10.0	10.0	8.7	7.7	6.8	8.4
B. L. J.	4.4	4.2	5.8	5.0	4.9	4.0	3.1		4.0

subnormal by as much as 10 mm.; but beginning at 5 per cent carbon dioxide the pressure again slowly increased until at 8 per cent of carbon dioxide it was 12 mm. above normal.

After a subject was restored to fresh air the hand volume usually did not change at once but it soon increased and ordinarily was back to normal within about 5 minutes.

BLOOD FLOW. Our attempts to determine the rate of blood flow have thus far been limited to the indirect methods, namely, those of Stewart (26) and of Hewlett and Van Zwaluwenburg (13), for estimating the flow through the hand; and the recoil board method of Henderson (11) for the output of the heart per beat. The data obtained with Stewart's hand calorimeter are given in table 4 and the curve of the average change in figure 2. The general tendency brought out by this method is a retardation in the rate of the flow of blood in the hands as the carbon dioxide of the inspired air increases, this retardation averaging 44.4

per cent at 7 per cent of carbon dioxide. Up to 2 per cent of carbon dioxide a slight increase in the flow may occur, but it ordinarily begins to lessen from between 2 and 3 per cent of carbon dioxide.

Similar results were obtained in 6 experiments with the Hewlett-Van Zwaluwenburg (13) method. In 3 cases the rate of flow increased up to 2 and 3 per cent of carbon dioxide, and then slowly decreased and continued to retard until at the end it was subnormal; in another case the flow gradually decreased throughout the entire experiment; while in the other two cases there was no clearly defined change, the rate fluctuating above and below normal throughout the entire period.

The observations with the recoil board were also made on six subjects. This method depends upon the Newtonian principle that "every action has an equal and opposite reaction." During a few seconds the subject holds his breath while a record is made of the recoil curves. The distance through which the body and board recoils is believed to afford an index of the relative size of the heart beats in an individual at different times. In figure 3 one of our records has been reproduced. In this experiment the records labeled N1 to N6 are the preliminary normals and N31 to N36 are post-experiment normals. There is a marked decrease in the amplitude in records 19 to 24 and a slight increase in 26 to 29 taken just before the subject was returned to fresh air. The evidence obtained from all the recoil curves indicates that the systolic discharge from the heart is not increased and may even be somewhat decreased. At least three of the experiments indicate a decrease, one of which is given in figure 3. The remaining records show recoil curves of quite uniform amplitude.

Our circulatory results obtained on men subjected to a gradual increase in carbon dioxide in the inspired air, up to 7 and 9 per cent, should be compared with other observations on the effects of carbon dioxide. Hill and Flack (14) have found man able to breathe 15.3 per cent of carbon dioxide. They studied the circulatory effects on cats and dogs, using from 10 to 30 per cent of carbon dioxide, and found that with moderate doses the blood pressure was raised, while the effect was most marked between 10 and 25 per cent. At these percentages they believed the vagus and vasomotor centers were stimulated and that at higher concentration the blood pressure fell, owing to a depressant effect upon the heart muscle. Kaya and Starling (21) in the spinal animal observed that carbon dioxide caused a rise in the arterial pressure and that a moderate excess did not injuriously affect the heart, furthermore it was thought that possibly the functional capacity might be increased.

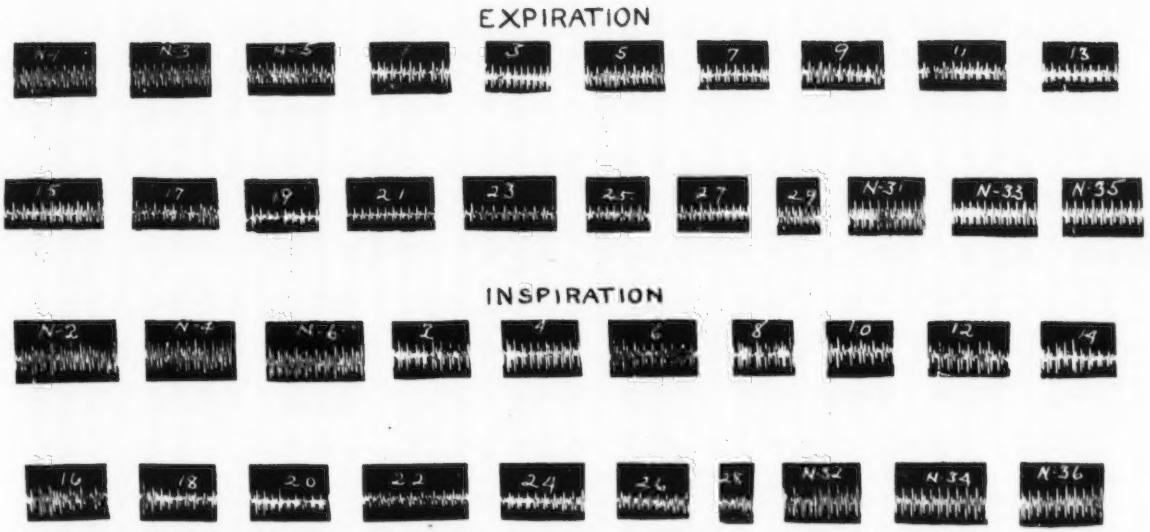

Fig. 3. Recoil board record during exposure to a gradual increase to 8 per cent in the inspired CO_2. The records were taken at regular intervals while the breath was held. *N*, normal before and after.

Jerusalem and Starling (20) with the heart-lung preparation showed that moderate percentages of carbon dioxide (2 to 8) increased the ventricular output. Itami (19) likewise found the cardiac output increased and concluded that a rise in blood pressure caused by small percentages up to 8 per cent is chiefly due to the increased action of the heart pump. Over against these observations are the experiments of Hooker (16) and Ketcham, King and Hooker (22) in which carbon dioxide was shown to relax vascular and cardiac muscle. It was pointed out that Jerusalem and Starling's results might be due indirectly to the effect upon the pulmonary vascular bed. Patterson (24) with the heart-lung preparation found that the administration of carbon dioxide caused a reduction in the amplitude of the heart, an increase of the diastolic volume and a slower rhythm, the result being a reduction of the minute volume. Campbell, Douglas, Haldane and Hobson (3), by determining the circulation rate by means of the carbon dioxide in arterial and venous blood, found that 5 or 6 per cent of inspired carbon dioxide while increasing the breathing about five times the normal did not appreciably influence the circulation rate. Our data obtained by means of the Henderson recoil board method for determining the relative size of the heart beats appear to support the Campbell, Douglas, Haldane and Hobson conclusion. We are continuing our study and hope to report on this point at another time. In the opposite condition in which carbon dioxide was quickly removed from the blood of animals by overventilation of the lungs, Dale and Evans (4) found the reduction in the heart output per minute was too small to be a serious factor in the fall of arterial pressure.

The lessened flow of blood that we found in the hand may be accounted for by the observations of Itami (19) and of Fleisch (8). In the intact animal Itami observed that constriction of the fore-limb vessels accompanied the general rise of blood pressure caused by carbon dioxide; and Fleisch in experiments on the hind legs of frogs perfused with Locke's solution found that carbon dioxide caused a dilatation with low concentrations (up to 3 per cent), while with stronger solutions a constriction became evident. He assumed that the dilator effect is due to action on a nervous component and the vasoconstriction to a direct action on the muscles of the arterioles. Dale and Evans (4) with cats and rabbits obtained an expansion of a normal limb with excessive ventilation of the lungs and a shrinkage again when respired air was inspired. They conclude that carbon dioxide has a specific stimulating action on the vasomotor centers in the brain and spinal cord.

Our data obtained with men indicate that carbon dioxide exerts a stimulating action, either directly or indirectly, on the vascular tissues in that with the rise in arterial pressure there is also a peripheral vasoconstriction indicated by the changes in hand volume and lessened flow of blood through the hand. It appears that the heart is also stimulated, since there is almost always some increase in the frequency of the heart beat. The rise in capillary and venous pressure which was also always present suggests an increased return of blood to the heart and, therefore, a well-defined increase in the minute volume of blood flow from the heart. Contrary to this indication we find the heart output per beat may sometimes be lessened and never clearly increased. The available evidence indicates that the minute volume is not appreciably altered. This then makes it difficult to explain the rise in the capillary and venous blood pressures. Henderson and Harvey (12) working with decerebrated cats observed that an accumulation of carbon dioxide in the blood caused an abnormally high venous pressure, a dilatation of the veins and an exaggeration of the volume of venous return to the heart, but that there was little or no effect upon the arterial pressure other than an increase in the amplitude of the pulse. They found, as we find for men, that the venous pressure develops gradually as the carbon dioxide accumulates in the tissues and again gradually falls as the carbon dioxide is ventilated out of the tissues. They believe that carbon dioxide acts directly on the venules, a decrease, as in acapnia, causing venule constriction and an increased venule dilatation. Dale and Evans (4) do not accept this explanation but attribute the changes to variations of the normal tone of the arterioles or of the capillaries or of both through the action of carbon dioxide on the vasomotor centers.

Respiration. Haldane and Priestley (9) have well shown that the respiratory center is extraordinarily sensitive to very slight increases in the carbon dioxide percentage of the alveolar air. A study of two men by use of a body plethysmograph and a box arranged over the head so that the carbon dioxide of the inspired air was allowed to rise to around 6 per cent, showed that the depth of breathing increased as much as 320 per cent and that the frequency rose from 14 to 27. The frequency of breathing was not changed until the carbon dioxide reached 3.2 to 4.8 per cent. Douglas and Haldane (6) found that the hyperpnea of a gradual increase in the inspired carbon dioxide develops smoothly and gradually. Experiments by Hill and Flack (14) on cats and dogs revealed an increasing excitatory effect up to 35 per cent, but above 35 per cent the carbon dioxide had a depressing effect upon the respiration.

Paul Bert (2) reported that death sometimes occurred when the carbon dioxide rose to 30 per cent.

By the method of rebreathing 30 liters of air in which the carbon dioxide gradually accumulated and the oxygen gradually diminished, Hough (18) found that the respiratory response of individuals differed, one subject having recourse chiefly to increased depth, another to increased rate, while a third might make use of both expedients. The dyspnea was almost invariably ushered in by an increase in the depth, and this was found to increase while the carbon dioxide rose to as much as 4 to 6 per cent. In the later stages a decrease in depth usually occurred which was always connected with an increase in rate. It was said that "in general, then, the rate and depth of respiration tend to vary inversely."

The alveolar air. In order that we might be certain that our subjects were under conditions comparable with those used by other workers we determined the alveolar carbon dioxide on a group of eight cases and compared our data with some of those recorded by Haldane and Priestley (9). Our determinations were all made on alveolar air at the end of inspiration by the Haldane and Priestley method. In order to establish the average condition for the group we first plotted the data for each man and from the resulting curve estimated the alveolar air percentage of carbon dioxide for each per cent of the carbon dioxide in the inspired air for the respective units 1 to 7. The average for the normal carbon dioxide content of the alveolar air under normal atmospheric air of 0.03 per cent carbon dioxide was 5.4 per cent and the average at each per cent of inspired air from 1 to 7 respectively was as follows: 5.6, 5.9, 6.2, 6.63, 7.2, 7.63 and 8.4. The method of establishing the curve necessarily gives greater accuracy after the inspired carbon dioxide reaches 2 per cent than it does from normal atmospheric air to 2 per cent carbon dioxide. However, if it be approximately correct it is evident that even a slight increase in the inspired carbon dioxide does influence the alveolar carbon dioxide and thus the arterial blood content of this gas. The curve of alveolar carbon dioxide increase rises gradually but with ever increasing increments after reaching 2 per cent in the inspired carbon dioxide. Haldane and Priestley have tabulated 3 experiments on 2 subjects which taken together show even a more gradual early rise, but these are correspondingly steep after reaching 4 per cent in the inspired carbon dioxide.

Minute-volume of breathing. Our results for the two groups of experiments, high and low oxygen, are summarized in table 5 in which the mean minute-volume and the percentage of increase are given. Accord-

ing to Hough (17) the minute-volume and rate of breathing are distinctly lower when with the same content of carbon dioxide the air contains 60 to 80 per cent of oxygen, than when the initial atmosphere is ordinary air. Our two groups reacted very much alike even though the available oxygen at 7 per cent of carbon dioxide for the low oxygen group averaged only 16.3 per cent, while some were as low as 11.8 per cent; and for the high oxygen group it ranged between 25 and 35 per cent. At 7 per cent of carbon dioxide the average increase in the breathing was 516.9 per cent for the former and 511.9 per cent for the latter group. In this our results agree with Campbell, Douglas, Haldane and Hobson (3) who find that the alveolar oxygen pressure can be varied within wide limits

TABLE 5

The mean minute volume of respiration

CARBON DIOXIDE	HIGH OXYGEN GROUP		LOW OXYGEN GROUP	
	Average respiratory volume in liters	Per cent of increase	Average respiratory volume in liters	Per cent of increase
per cent				
0.03	6.30		7.53	
1.0	8.29	31.6	9.33	23.9
2.0	11.30	79.5	11.30	50.0
3.0	15.61	147.8	14.14	87.8
4.0	19.44	207.9	18.79	149.5
5.0	25.70	307.9	24.05	219.4
6.0	32.67	418.6	32.89	336.8
7.0	38.55	511.9	46.47	516.9
8.0	46.60	639.7		

without sensibly affecting the excitability of the respiratory center to carbon dioxide. In another type of experiment Benedict and Higgins (1) found that oxygen-rich gas mixtures have no influence on respiration.

The minute-volume ordinarily increased within the first minute or two of rebreathing. In the 52 experiments with a high content of oxygen there were only 5 times in which there was no evidence of increased breathing at 1 per cent of carbon dioxide; and in the 20 experiments, in which the oxygen gradually decreased as the carbon dioxide increased, there were 2 times in which the response had not begun at 2 per cent carbon dioxide and 2 more in which it was not evident at 1 per cent. At 1 per cent carbon dioxide the high oxygen group had an average increase of 31.6 per cent and the low oxygen group an increase of 23.9 per cent. Zuntz (27) found that the presence of 1 per cent of carbon dioxide in-

creased the minute volume by more than 20 per cent; while Scott (25), working with decerebrated cats, obtained an increase of 23 per cent at 1 per cent carbon dioxide.

In every one of our cases the minute-volume increased steadily to the end of the experiment. The maximum increase of 1052 per cent was found in a man who had a normal ventilation of 7.13 liters that was raised to 75.0 liters at 8 per cent of carbon dioxide. This subject's pulse rate rose from 95 to 110, the systolic arterial pressure from 102 to 152 mm. and the diastolic pressure from 68 to 90 mm. The nearest approaches to this record were from 7.8 to 560 liters, 717 per cent, and from 7.6 to 540 liters or 711 per cent increase.

The frequency of respiration. The changes in the rate of breathing have been carefully followed in 20 cases to determine the individual differences. The average normal rate of breathing ranged between 8 and 18, average 12.7 breaths per minute. As the carbon dioxide increased in the inspired air the average frequency at each increase from 1 to 8 per cent respectively was as follows: 12.8, 14.4, 15.1, 17, 17.9, 19, 21.6 and 22.6. These are individual differences, but we have not found them necessarily constant. There is a tendency for women to make the compensations for the minute-volume increase more by an increasee in the rate than in the depth of breathing. Thus in certain instances the depth of breathing was not altered until 3.5 or 4 per cent carbon dioxide had been reached, while the rate gradually increased from the very first minute. Of the 20 cases the frequency first gave an increase as follows; at 1 per cent, 3 cases; at 2 per cent, 7 cases; at 3 per cent, 2; at 4 per cent, 3; at 5 per cent, 3; at 6 per cent, 1; and at 7 per cent, 1. The frequency, therefore, was already increased in 60 per cent of cases before 4 per cent of carbon dioxide had been reached.

Not once did the rate fail to increase, the increase at 7 per cent carbon dioxide ranged from 2 to 18 breaths per minute, or from 17 to 200 per cent.

The depth of breathing. Almost all of our data were obtained from persons accustomed to serving as subjects of experimentation, hence there is little or no evidence of psychic disturbances in the rate and depth of breathing. The normal depth ranged between 410 and 780 cc., with an average of 570 cc. The ordinary response in the depth of breathing to carbon dioxide is shown in the following average depths for a group of 20 experiments determined for the respective percentages of of carbon dioxide from 1 to 8: 630, 780, 1000, 1150, 1480, 1760, 1920, 1820 cc; an average increase at 7 per cent carbon dioxide of 337 per cent.

In all but 6 experiments the depth of breathing was already somewhat increased at 1 per cent carbon dioxide, in 4 of these the response was delayed to between 2 and 3 per cent, to between 3 and 4 per cent in one and between 4 and 5 per cent in the other. In those instances in which the depth of breathing had not increased at 1 and 2 per cent of carbon dioxide the frequency of breathing had already definitely accelerated so that the minute-volume was increased. In one experiment the rate in 9 minutes, up to 3.2 per cent carbon dioxide, increased from 9 to 16 breaths per minute; the depth of breathing at the beginning was 622 cc.

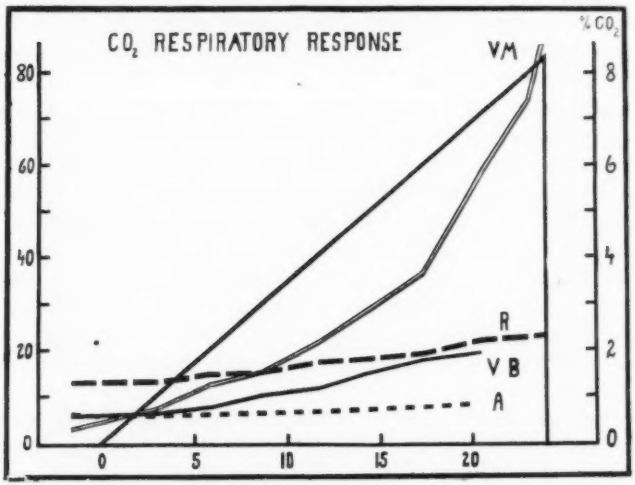

Fig. 4. Curves of means. *VM*, minute-volume; *VB*, volume per breath; *R*, frequency; *A*, per cent of alveolar CO_2.

and in the ninth minute 619 cc.; while the minute-volume during this time rose from 5.7 to 9.9 liters. From then on both the rate and depth gradually continued to increase.

There is of course a limit to the depth of breathing. When this is reached further recourse for increasing the per-minute volume is limited to a greater use of the increase in the frequency of breathing. So apparently in 7 experiments the maximum depth of breathing was reached at 6 or 7 per cent of carbon dioxide after which the further increase in per minute volume was made by hurrying the breathing, and in 4 of these toward the end of the experiment the depth decreased somewhat as the breathing became more rapid. The inverse relation-

ship between the rate and depth of respiration reported by Hough was not as a rule observed among our cases. In figure 5 are reproduced typical kymograph records of the three types of respiratory response observed. The first is from a case in which the compensation was largely made by an increase in rate, the second in which it was made in the depth of breathing, and the third in which a combination of increase in rate and depth was made. The relations of the several respiratory changes have been brought out in figure 4 by plotting the data with respect to time and the percentage of carbon dioxide.

Chest girth. It was noticed when listening to the heart sounds during the period of rebreathing that the pulmonic and aortic heart sounds grew less distinct as the breathing deepened and that the ribs appeared to be held more nearly horizontal than usual. So chest measurements were made throughout 12 experiments. The circumference determined at the end of expiration over the nipples was increased as the experiment proceeded by from one-half to one and a half inches, the increase averaging about an inch. Furthermore the return to the original size after the close of the experiment was slow, never within the first 10 to 20 minutes and apparently sometimes not for several hours. Some subjects felt that it was impossible to exhale completely, or that the succeeding inspiration began before exhalation was satisfactorily completed. The after-effect on the chest circumference suggested an increased tone of muscle, but there was no evidence of such tone in other than the chest muscles.

The time at which each of the several circulatory and respiratory factors is first affected and the amount of response are well shown by an experiment on one subject, E.C.S., which is given in detail in figure 6. The systolic, S, diastolic, D, and pulse pressures, PP, were unchanged until the fifth minute (1.5 per cent carbon dioxide) when each began a steady rise but at different rates, with the systolic rising most rapidly and in greatest degree. The pulse rate, P, did not clearly accelerate until the 19th minute (about 5.8 per cent CO_2). The minute-volume, V, of breathing increased from the beginning, while the frequency, R, of breathing did not definitely increase until the 18th minute (5.5 per cent CO_2). The depth of breathing clearly increased from the beginning and was responsible for the increase in the minute-volume. In this experiment the respiratory response to carbon dioxide is in evidence before the vasomotor system or the heart are stimulated. The order and time at which the several factors responded in this case are typical for men generally.

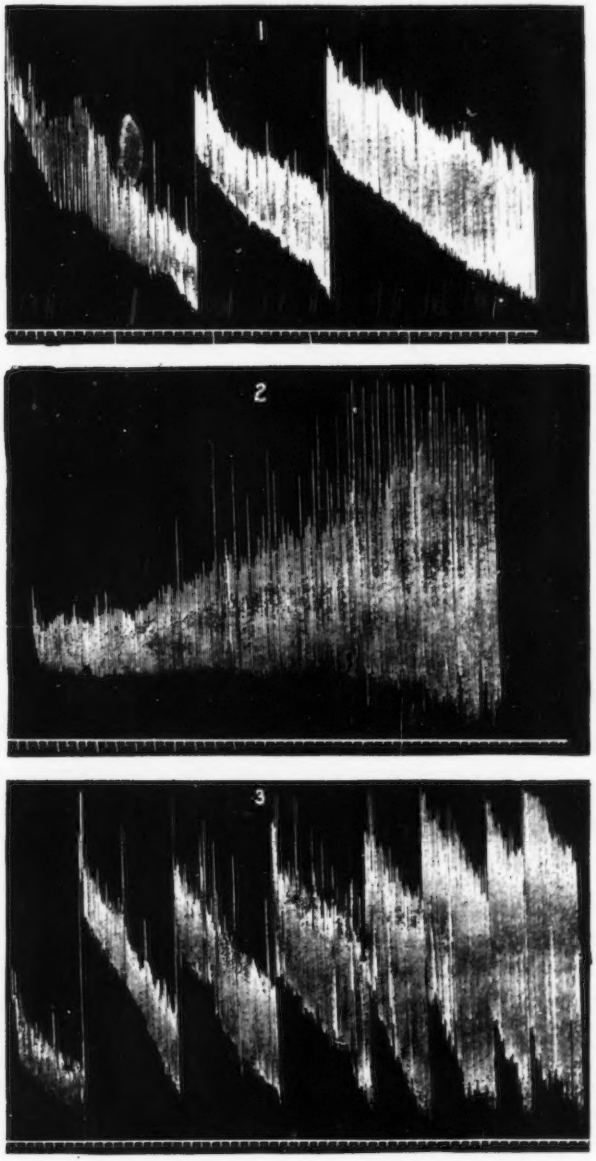

Fig. 5. Types of respiratory response

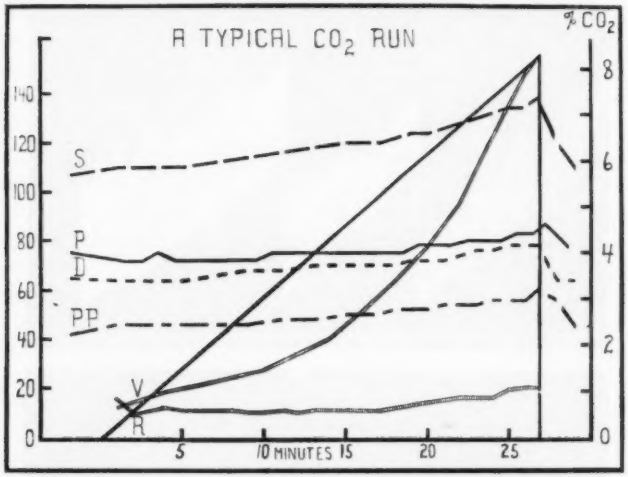

Fig. 6. Subject E. C. S. Final CO_2, 8.4 per cent. *S*, systolic pressure; *D*, diastolic pressure; *PP*, pulse pressure; *P*, pulse rate; *V*, minute-volume of breathing; *R*, frequency of breathing.

SUMMARY

1. The effects of a gradual increase in carbon dioxide have been determined in two types of experiments, in one the oxygen was maintained at about 30 per cent and in the other the oxygen decreased as the carbon dioxide accumulated in the inspired air.

2. The pulse rate usually first accelerated at about 5 per cent carbon dioxide, sometimes as early as 1 per cent. An after-effect usually was present for 5 to 7 minutes.

3. The systolic, diastolic and pulse arterial blood pressures always increased, ordinarily beginning to rise at between 2 and 4 per cent carbon dioxide. The systolic pressure rises most rapidly, but the diastolic rise is frequently seen first. The after-effect is fleeting.

4. The capillary blood pressure rises steadily as the inspired carbon dioxide increases and shows no after-effect.

5. The venous blood pressure always rises and begins to do so as early as 1 per cent of carbon dioxide. An after-effect of from 5 to 10 minutes occurs.

6. The changes in hand volume were an increase up to about 3 per cent carbon dioxide, followed by a gradual decrease to below normal.

7. The blood flow through the hand decreased as the inspired carbon dioxide increased, while the minute-volume from the heart was not materially altered.

8. The minute-volume of breathing increased gradually and smoothly from the very beginning of the accumulation of carbon dioxide in the inspired air. The volume per breath usually increased as early as the minute-volume.

9. The frequency usually increased later than the depth of breathing, but had accelerated in 60 per cent of all experiments before 4 per cent of carbon dioxide was reached.

We wish here to express our appreciation of assistance given by Major L. H. Bauer.

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